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THE PHYSIOLOGY OF TWINNING

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THE PHYSIOLOGY OF TWINNING

By

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PREFACE

This book deals primarily with the causes and consequences of twinning. In 1917 was published a volume belonging to this series and written by the present author, entitled *The Biology of Twins*. That volume was limited to twinning in mammals and was largely morphological in character. The word "twins" was viewed broadly so as to include one-egg (monozygotic) as well as two-egg (dizygotic) twins. We now realize that true twinning is essentially a matter of the division of a single egg at various stages of development and that dizygotic twinning is only a case of plural offspring born simultaneously. The present volume is confined to one-egg twinning and, instead of being limited to mammals, includes all known types of complete or partial one-egg twinning in the animal kingdom. In *The Biology of Twins* very little attention was paid to theories as to the causes of twins or to the interinfluences of twins upon each other. One definite theory, however, was advanced by the writer to account for the curiously unique process of twin-formation in the armadillos. This theory was the first real causal theory of twinning based on facts. Inasmuch as this theory is now supported by a great body of evidence from many sources, the writer, at the risk of appearing too solicitous about matters of priority, feels impelled to lay definite claim to its authorship. This theory is the center and substance of the present book. When used as a working hypothesis it bids fair to explain a long array of peculiar twinning processes in diverse

groups of animals. A considerable number of interesting situations, such as hemihypertrophy in man, bilaterality in echinoderm larvae, symmetry reversal, double limbs and tails, supernumerary organs, and certain types of tumors, turn out to be phases of twinning. This all seems to mean that twinning is a much more general phenomenon than we have previously supposed. Whether it occurs in worms or in man, it expresses itself in the same series of types; and there is a deep-seated coherency and consistency about its varied expressions. It is because twinning is believed to be a consequence of one of the most fundamental of biological processes that the present volume has been written, in the hope that some of the riddles of life may at least partially be answered.

I wish to express my thanks to my friends, Professor F. R. Lillie and Dr. A. W. Bellamy, for their help in reading the manuscript and for valuable suggestions, and to Mr. Kenji Toda, who has so skilfully drawn or redrawn the illustrations.

H. H. Newman

April 10, 1922

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CHAPTER I

THE NATURE, SCOPE, AND CAUSES OF TWINNING

THE NATURE OF TWINNING

Strictly speaking, twinning is twaining or two-ing—the division of an individual or an organ into two equivalent and more or less completely separate individuals or organs. The term dichotomy is almost a perfect synonym for twinning, for it means literally a process of division into two parts. When, as in the specific twinning of the armadillos and in the sporadic twinning of other species, more than two offspring are produced from one egg, the condition may be still called twinning because the process of dichotomy is simply repeated two or more times. Thus we may have single twinning, double, triple, or even quadruple twinning. The most advanced phase of twinning is that seen in the South American armadillo *Dasypus hybridus*, which undergoes always as many as three twinning processes and often proceeds well into a fourth, so as to produce up to twelve embryos from a single egg. I do not consider polyembryony as exhibited among the parasitic hymenoptera an instance of true twinning: this, for reasons that will be made clear later. Furthermore, since twinning is essentially a division of one into two, we are not justified in retaining within the category of twins any cases in which two individuals have originated from two germ cells originally separate. In this narrower sense, therefore, twinning proper is always monozygotic or one-egg twinning.

Though thus limited, twinning is still a multifarious process: there are many kinds of true twinning. If the blastomeres of the two-cell stage of cleavage, destined to develop into the right and left primordia of a single individual, become physically or physiologically isolated so as to develop independently of each other, two half-sized, but otherwise normal, individuals result: these are twins. If a young blastoderm becomes bilaterally separated into two more or less equivalent blastoderms and two independent individuals result, these are twins. If a single blastoderm loses its axiate organization so that two equivalent secondary points of gastrulation arise instead of one, and two embryonic axes result, this is twinning. If a single embryonic axis exhibits a tendency for the two bilateral halves to grow independently and a double-headed or double-tailed individual results, this, no matter how complete or incomplete the isolation of the two sides, is twinning. If an appendage such as a hand or a foot becomes double instead of single, this is also a phase of twinning.

Twinning is a matter of great definiteness and depends on the bilateral organization of the embryo. Rarely does a division into more than two individuals occur, unless one of the two redivides. Triplets are a hundred times as rare as are twins. This very fact emphasizes the essential feature of twinning: its two-ness. Moreover, not merely two individuals, but two *equivalent* individuals are formed, which are nearly always symmetrically placed with reference to each other. Neither one is the original individual; neither one is secondary or subordinate to the other; but both are equivalent individuals regenerated from a half-embryo.

Twinning is essentially a process of regulation or regeneration of a whole individual out of a prospective half individual. All that is necessary in order to get two individuals instead of one is to accomplish the division of an embryo into two *equivalent* regions. Each region is totipotent and soon reorganizes its own complete axiate relations and becomes a whole individual. When the separation of halves is incomplete, we get, in the case of whole organisms, double monsters; and in the case of special organs, merely double organs.

THE DISTRIBUTION OF TWINNING THROUGHOUT THE ANIMAL KINGDOM AND ITS SIGNIFICANCE

In his book, *Materials for the Study of Variation*, Bateson surveys the incidence of double monstrosity in the animal kingdom. We find that it is found most commonly among vertebrates, not infrequently among annelids and arthropods, occasionally in echinoderms of various classes, rarely in coelenterates, cestodes, brachiopods, protozoans. I have never seen a reference to a case of twins or double monstrosity in Mollusca, Nemertinea, Nemathelminthes, Rotifera, Ctenophora, or Tunicata. These latter groups are characterized by *determinate cleavage* in its most definite form. In such groups the uncleaved egg is already highly organized, each part of the egg having a definite prospective value as an organ-forming region. Hence, if the blastomeres are isolated in two-, four-, or eight-cell stages they are not able to produce whole individuals, but merely parts of individuals. If divided in the two-cell stage, one cell will develop into a left-half and the other cell into a right-half embryo. It is no wonder then that in groups

with strictly determinate cleavage we find no examples of twinning, for twinning requires a totipotency of blastomeres or regions of the blastoderm.

Equally significant is the fact that twinning is especially characteristic of the vertebrates, a group in which cleavage is most clearly *indeterminate*, in the sense that the early cleavage cells appear to be totipotent, i.e., each able, if isolated, to produce a whole individual. In the vertebrates, although the axes of polarity and symmetry are already laid down in the undivided egg and the axes of the embryo tend to coincide with those of the egg, it appears to be relatively easy to break down the axiate relations either in the egg or in various embryonic stages, and then to establish new axes. This all means that, apart from their axiate integration, the blastomeres, until a relatively late period of cleavage, are potentially equivalent, no blastomere containing any unique organ-forming material, but each being essentially germinal in character and able under the proper conditions to become a new apical point and to establish new axes of polarity and symmetry. This extreme versatility of the elements of the vertebrate blastoderm is, in my opinion, the secret of its twinning capacity. There is a good reason then why the vertebrates are par excellence the favored group for twins.

The other group in which twinning occurs readily is that of the echinoderms, in which cleavage is also decidedly indeterminate. In the annelids and arthropods, where partial twinning occurs only occasionally or is confined to a few types, we find that cleavage is strictly intermediate between the extreme determinate and the extreme indeterminate types. Thus again the parallel-

ism between the mode of cleavage and the incidence of twinning is confirmed.

THE CAUSES OF TWINNING

For a long time I have held the view that twinning is essentially a phenomenon involving a physiological isolation of equivalent parts of the blastoderm and a regulation of the isolated or twinned regions into complete embryos. As to the cause of physiological isolation, I have maintained, for the armadillo at least, that the essential feature is temporary cessation or radical retardation of development at a critical period. It is not especially important for the theory to determine the exact cause or causes of the lowered developmental rate, for we know that a great many agencies give the same end-result so long as they accomplish a retardation. What retardation evidently does to the egg or embryo is more or less completely to disorganize its integrational relations, or, in a word, to deaxiate it. If through retardation the polarity of the egg or embryo becomes so weakened that there are no relatively high or low metabolic regions, all parts of the embryo are left on a parity; so that, when normal developmental conditions once more appear, any part of the embryo may become the apical point and a new gradient will be established. What usually happens, however, is that enough of the original axis of polarity persists to permit just two equivalent points on the axis, at equal distances from the original apical point, to remain in such a condition that they are favored when developmental conditions return, and these become the growing points or apical ends of twin embryos. Very commonly the developmental re-

tardation does not seriously affect the blastoderm until the process of gastrulation begins, a process of extreme delicacy, as has often been pointed out. Once fairly started the cleavage process seems to be easily accomplished even under great difficulties. This was clearly brought out by the writer (Newman, 1915) in his work with teleost hybrids.

Almost any cross, even those between the most distantly related species, will go through cleavage normally or nearly so, but, because of the relative slowness of the cleavage process, the important crisis of gastrulation is the usual halting-point for such hybrid embryos: their developmental momentum is insufficient for them to accomplish gastrulation. Now gastrulation results in the establishment of the embryonic axes, including the axis of polarity and the axis of symmetry. The result is that if gastrulation is only temporarily halted the process of formation of the axis of symmetry, or the bilateral axis, may be so interfered with that the primordia of the two equivalent halves of the symmetrical embryonic axis may become more or less completely isolated physiologically, and each half may produce a more or less perfect whole embryo in the region where the isolation has occurred.

INTERPRETATION OF DOUBLE MONSTERS (*COSMOBIA*)

My interpretation, therefore, of double monsters, at least of those of a symmetrical sort which have certain of their central or axial structures united, is that they are products of a partial twinning process involving a separation of equivalent right and left parts of an originally single embryonic axis. This is opposed to the

prevailing theory that double monsters are products of the fusion of two originally complete and separate embryos. This question is quite crucial for our general interpretation of the nature and causes of twinning. It is a time-honored question and has been discussed pro and con by leaders in the history of zoölogy for nearly two hundred years. Important names such as those of Caspar Frederick Wolff, Meckel, the two Saint-Hillaires, Knoch, Dareste, Rauber, Panum, and others are associated with the earlier phases of the problem. Within recent times we find such men as Windle, Gemmill, Kaestner, Wilder, Stockard, and others still taking sides on the vexed question of whether double monsters are derived from the division of one or the fusion of two embryonic axes. Gemmill and Stockard, among recent writers, stand for the fusion of separate embryos; Wilder and myself, on the other hand, hold to the view that double monsters are incompletely divided single embryos. All of these views will be discussed in detail because, to my mind, an acceptable theory of the morphology and the physiology of twinning depends on a correct interpretation of the mode of origin of double monsters.

If double monsters are merely the incompletely separated and regenerated bilateral halves of an originally single embryo, we have a rational interpretation of *situs inversus viscerum* and mirror-image symmetry. From this point of view we can proceed to an understanding of various kinds of partial twinning and of such allied phenomena as symmetry reversal in various asymmetrical forms such as echinoderm larvae. The conception that double monsters are products of a partial

fission is an integrating thread running through the whole fabric of our twinning theories.

THE ORDER OF PRESENTATION OF DATA ON TWINS

In general, the order of chapters is based on the relative simplicity or the fundamental character of the twinning processes. The simpler and more fully understood cases of twinning, such as those in the starfishes and earthworms, come first because it is believed that there the phenomenon of twinning is less obscured by developmental complexities. The most obscure and least understood cases of twinning, as that in man, are reserved for later consideration in order that we may have as much information as possible about the modes, causes, and consequences of twinning before tackling the most difficult of our problems. In dealing with twinning in the vertebrate classes the phylogenetic order is followed because this order seems to lead from the simpler to the more complex phases of the subject. The causes of twinning in the armadillos of the genus *Dasypus* seem to me to be more definitely known than those of any other vertebrate, but the restatement of this situation is postponed until after the consideration of the twinning conditions in the birds and in the reptiles.

The study of the literature on twins and double monsters in man brought to light two rather unexpected and extraordinary situations that seem to me to throw much light on matters formerly quite obscure to biologists. The first of these situations has to do with the various developmental hazards of human twins, especially one-egg twins, due largely to interinfluences of the two

individuals. The second situation, which is technically called hemihypertrophy, or a unilateral gigantism of one half of the body, strongly suggests partial physiological isolation of the bilateral primordia, or minimal twinning. The consideration of double monsters of all classes has brought to light the existence of the phenomenon known as *situs inversus viscerum* and other sorts of mirror-image symmetry between the two components. This phenomenon strikes deep at the roots of the physiology of symmetry and asymmetry in the vertebrates; hence a chapter is devoted to an analysis of the observed conditions. After the subject of twinning of whole organisms has been concluded the discussion shifts to twinning in tails and limbs. Duplicity in tails turns out to be merely a phase of bilateral twinning involving a posterior growing region, the tail-bud. Twinning in appendages, however, seems to involve certain complexities that are not present in bilateral twinning. An understanding of the sources of complexity, however, seems to indicate that the same fundamental causes and consequences of twinning hold good for twinning or duplicity in limbs as for twinning of the whole body. There occur, moreover, comparable phenomena of symmetry reversal and mirror-imaging that seem to aid in an analysis of the general laws of symmetry.

In a final chapter twinning as a mode of reproduction is discussed. It is shown that twinning is a form of axiate reproduction, that twinning is not a reminiscence of a lost ancestral asexual phase in an ideal alternation of generations, and that twinning and polyembryony are two entirely different phenomena and should never be confused.

Throughout the book an attempt is made to put the various types of twinning in their proper place in the system of biological phenomena and to show in what ways a knowledge of the causes and consequences of twinning helps to advance our knowledge of several obscure and difficult but none the less fundamental aspects of animal biology.

CHAPTER II

EXPERIMENTAL PRODUCTION OF TWINS IN STARFISHES

INTRODUCTION

Since *The Biology of Twins* was written much new light has been thrown on the nature and causes of twinning and perhaps the clearest analysis of the fundamentals of twinning has been obtained through the experiments of the present writer on the eggs and embryos of the Pacific Coast starfish, *Patiria miniata*. It was felt that an experimental analysis of twinning was almost impossible in the case of mammals and it was therefore decided to make use of some species whose development was well known to be simple, readily controlled, and capable of easy observation. One would naturally select for such work either the bony fishes or the echinoderms: two groups which for a long time have been favorite materials for experimental biology. The echinoderms were chosen in preference to the fishes partly because the processes of cleavage and gastrulation are simpler and less open to controversy. For a long time I have felt that twinning was intimately associated with the process of gastrulation and on that account it seemed wise to study twinning in a form in which this whole process is so nearly diagrammatic.

When I went to the Pacific Grove laboratory in the spring of 1920, one of my chief concerns was to study experimentally the process of twinning in some echino-

derm, but I hardly expected to find so favorable a form as *Patiria*, a form that produces abundant twins almost without any effort on the part of the investigator. Under ordinary laboratory conditions, and especially under conditions of crowding, one gets a high percentage of twins of many sorts. It then becomes the task of the investigator to discover the factors responsible for this high incidence of twins. Such an analysis has been made and is here to be presented.

THE CONDITIONS UNDER WHICH TWINS ARE PRODUCED

A. *Parthenogenetic twins.*—It was a matter of some surprise to note in certain control cultures of *Patiria* eggs, that had never been fertilized, considerable numbers of actively swimming larvae. Many of these larvae were of dwarf size, others were almost formless, wrinkled ciliated masses; but there were always some distinctly twinned larvae, in the sense that they had two or more archentera or primitive alimentary tracts. Such twins lived a long time but were always quite subnormal with respect to the development of typical larval characters. The percentage of parthenogenetically developing eggs in *Patiria* ranges from 0 in some cultures to as high as 75 in one culture. The usual percentage ranges from 1 to 10 and is more commonly about 2 or 3. Of the eggs developing parthenogenetically as many as a third, or possibly a half in some cases, show some phase or other of twinning.

What is there about parthenogenesis that favors twinning? This was, of course, the most obvious question that presented itself, and it was not to remain

long unanswered. One needed only to watch carefully a batch of unfertilized eggs simultaneously with another batch of fertilized eggs to note certain obvious differences: (*a*) Both sets of eggs undergo maturation at the same time. (*b*) Numerous eggs in the fertilized lot produce well-elevated fertilization membranes; while in parthenogenetic cultures this happens in only a few eggs. (*c*) In two hours after insemination the fertilized eggs begin cleavage, which goes forward at a rapid rate; while in the unfertilized lot those eggs that had formed a membrane fail to cleave and disintegrate after a few hours. (*d*) Some of the maturated eggs in the unfertilized lot, without having formed any distinctly lifted-up fertilization membranes, begin cleavage after about six hours, nearly four hours later than in the fertilized eggs, and cleavage goes on relatively much more slowly than in the latter.

It is obvious from these observations that the development of parthenogenetic eggs has been greatly slowed down. The stimulus afforded by the entrance of the spermatozoön has been lacking, as is evidenced by the absence of cortical changes and the failure of a fertilization membrane to be elevated from the vitelline membrane. Moreover, all of the developmental changes go on at an abnormally slow rate.

B. *Hybrid twins.*—Twins of another sort, rather better developed and more viable than parthenogenetic twins, were frequently found in cross-bred cultures in which the eggs of *Patiria* had been fertilized by the sperm of various other species of starfishes and sea urchins. As compared with pure-bred *Patiria* larvae, hybrid larvae always showed a very distinctly slower develop-

mental rate at all stages. It seems obvious, however, that though foreign sperm less completely stimulates the egg than own sperm, yet some stimulus is given, for hybrid embryos develop more rapidly and more normally than do parthenogenetic embryos.

C. *Twins due to crowding.*—Varying percentages of twins of an even more advanced type were frequently found in control batches of eggs that had been fertilized by own sperm, but had been allowed to remain in much too large numbers in relatively small containers. Some of the most advanced twin larvae were discovered under these conditions, and such twin larvae, when transferred to new quarters, where they had plenty of room and oxygen, developed farther than any other twin larvae observed. In some cases they practically recovered the single condition through the absorption of the smaller twin by the larger. It seems obvious that these larvae in the crowded cultures had suffered a period of growth depression due to the low oxygen and high CO_2 content of the sea water. It was my intention to perform some experiments involving lack of oxygen, but the work was interrupted before there was opportunity to do so.

Retarded development, the primary cause of twinning.—In all three of these methods of twinning the common factor is undoubtedly a retardation, more or less severe, of the normal rate of development. This was not at all an unlooked-for result; quite the contrary. One who, like the writer, had for years realized the fact that most, if not all developmental anomalies, are, in last analysis, the result of interferences with the normal developmental rate of organisms, and who had offered a theory of arma-

dillo twinning based on the idea that twinning was the direct result of interrupted development, would naturally be on the lookout for just this type of result.

THREE TYPES OF ONE-EGG TWINS

From the point of view of their mode of origin three distinct types of twins were readily distinguishable: (1) dwarf larvae resulting from the physiological isolation, followed by physical isolation, of the cleavage products of separate blastomeres; (2) double, triple, or multiple individuals resulting from the formation of two or more archentera; (3) "two-headed" larvae resulting from a dichotomy of the anterior end of the archenteron.

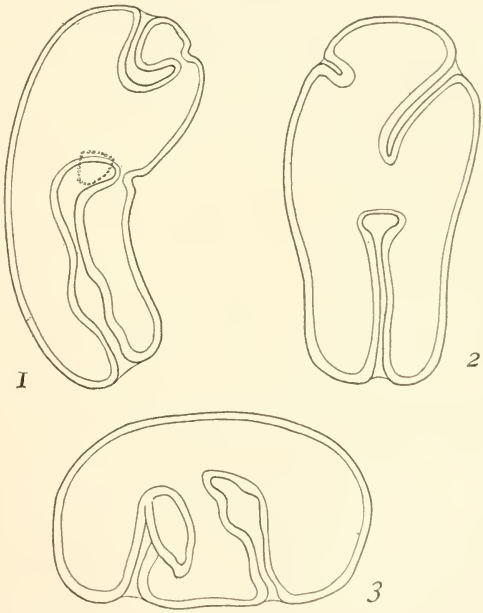
In addition to these three primary types certain secondary types appeared as the result of fusion of adjacent archentera to form individuals with a Λ-shaped archenteron, single in front and divided behind (Fig. 5). Each of these types will be discussed separately.

1. *Dwarf larvae.*—These larvae always appeared in parthenogenetic cultures. When the very much belated process of cleavage begins, it proceeds with so little energy that many eggs start the first cleavage and lose their developmental momentum before the two daughter-cells are fully separated. In others the first cleavage completes itself, but the second cleavage takes place in only one of the blastomeres. The belated blastomere either remains permanently undivided or else resumes division at a slower rate than the first. There is obvious physiological discoördination between the two sets of cells thus produced. Each produces a blastula, one less normal than the other, and before long they rupture the membrane and become swimming, half-sized larvae.

Neither one is fully normal, but both at least begin gastrulation and some of them form fairly normal gastrulae. Never, however, do these dwarfs attain a condition that approximates that of a bipennaria larva. They all stop at the gastrula stage. We may denominate this mode of origin of twins *physiological blastotomy;* for it is the result of the physiological isolation of the first two blastomeres, so that they act as though they were independent eggs. Such a condition is equivalent to the occurrence of two separate blastoderms on a fish or a chick egg.

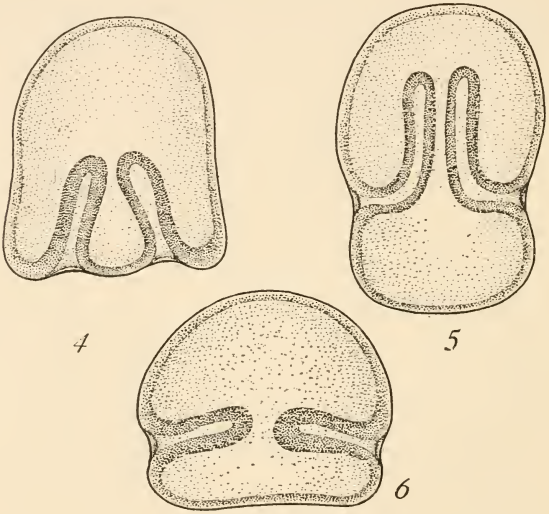
2. *Twins with two or more archentera.*—Much the commonest mode of twinning in the starfish is one in which two or more points of invagination occur, resulting in two or more archentera. Many varieties of this type of twin occur. A very common type is that in which the original archenteron seems to persist and a secondary one (often two or three) arise at the opposite or apical end of the larva. Such a larva may subsequently rid itself of these accessory archentera by closing their blastopores and pinching off the small archentera so as to leave one or more small internal vesicles or cysts that remain attached to the body wall for some time, only to be ultimately resorbed. Some biaxiate larvae, however, such as that shown in Figures 1 and 2, have the secondary archenteron nearly as large as the primary and the animal exists as a biaxiate bipennaria for a long time. In swimming, such a larva moves in a direction determined by the position of the primary blastopore, i.e., with this structure posteriorly directed. The anterior component of this twin seems to be under the dominance of the posterior or primary component.

Another common type of twin larva is one in which there seems to have been a partial obliteration of the primary symmetry relations and two secondary equivalent symmetries result. In such larvae both archentera develop symmetrically and are quite equivalent, so that it would not be possible to call one the



FIGS. 1–3.—Outline drawings of three types of twin larvae of the starfish *Patiria miniata*, showing plural archentera. Fig. 1, an advanced bipennaria with primary archenteron at the original basal pole and a well-developed secondary archenteron at the site of the original apical pole. Fig. 2, a similar bipennaria with two supernumerary archentera, a secondary and a tertiary. Fig. 3, a true twin larva with two symmetrically placed archentera, neither of which is the primary one. (From Newman.)

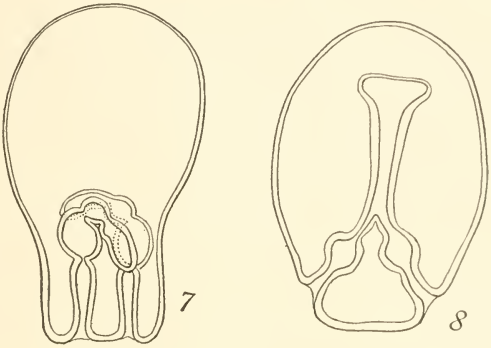
primary and the other the secondary component. Such twin larvae develop symmetrically throughout their lives and reach fairly advanced stages. Typical examples of the relations of the two components are shown in



FIGS. 4–6.—Three very common types of symmetrical twin larvae of *Patiria miniata*. Scores of such twins were found in almost every batch of larvae which were in any way retarded in development. (Original.)

Figures 3, 4, 5, 6, 7. When in larvae of this sort one of the archentera is originally smaller or secondarily comes to be less active in its growth than the other, it quite commonly happens that the smaller one comes into contact with the larger, fuses with it at its anterior end, and, after closing its blastopore, becomes first a pouch of the larger archenteron and then merely a thickening in the wall of the latter. This is a very

striking example of the way in which a stronger twin may dominate over and ultimately rid itself of a weaker twin so as to come to be practically a normal individual. Occasionally also two quite equivalent paired archentera grow toward one another at such an angle that they meet



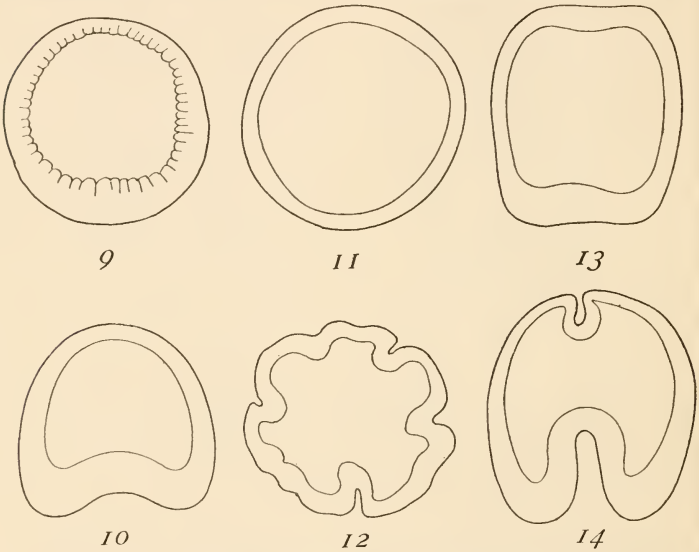
FIGS. 7, 8.—Two advanced larvae of *Patiria*. Fig. 7, a symmetrical twin with the two separate alimentary tracts considerably differentiated and exhibiting striking mirror-image symmetry. Fig. 8, a twin of the same age with the anterior ends of the two alimentary tracts fused to form a common pharynx. (From Newman.)

in the middle of the cleavage cavity and fuse at their anterior ends. Such larvae come to have one anterior and two posterior archentera and two blastopores. In several cases such larvae as this (Fig. 8) went so far as to differentiate two stomach enlargements, two oesophagi and two intestines, communicating with a common pharynx.

3. *Twins with bifurcated archenteron.*—A relatively rare type of retarded larva undergoes dichotomy of the anterior end of the archenteron, which results in a sort of "double-headed" condition. This phenomonen is

discussed in a subsequent section under the caption *An adidymi among starfishes* (pp. 23, 24).

The mode of origin of these twins resulting from plural gastrulation processes is of considerable interest. One can easily trace the twinning back to the blastula



FIGS. 9–14.—Types of blastulae with appropriate gastrulae of *Patiria*. Fig. 9, a typical blastula, with the typical gastrula (Fig. 10) that arises from it. Fig. 11, a blastula that has entirely lost its polarity, with the very abnormal multiple gastrula (Fig. 12) that arises from it. Fig. 13, a bipolar blastula, the original polarity of which has been only partially lost, with the bipolar gastrula (Fig. 14) that arises from it. (From Newman.)

stage. A normal starfish blastula has a very definite primary polarity. As shown in Figure 9, the blastula wall is much thicker on one side than the other. The cells of the thicker region are loaded with yolk granules

and those at the thinner pole are free from yolk. Obviously then we have a well-defined animal and vegetal pole, and gastrulation or archenteron formation is an ingrowth or invagination of the vegetal pole (Fig. 10). In the case of parthenogenetic blastulae, however, numerous deviations from the normal conditions are observed:

a) Some blastulae remain nearly solid, showing scarcely any cleavage cavity. Such blastulae never gastrulate at all.

b) Other blastulae (Fig. 11) are without any polarity. The cleavage cavity is large but the yolk material is evenly distributed among all of the cells. Such blastulae usually undergo multiple gastrulation, the surface invaginating intricately as in Figure 12.

c) Other blastulae, instead of having one thickened, yolk-laden region of the blastoderm, have two or more such regions (Fig. 13). Such bipolar and tripolar blastulae invaginate at two or three places to make the types of larvae in which there are two or three archentera. A typical bipolar gastrula is shown in Figure 14. If the original polarity is retained to some extent and only a relatively small, thickened, yolk-laden area appears at the opposite pole, we get gastrulae and bipennariae of a bipolar sort with a supernumerary archenteron at the original apical end. Sometimes the thickening at the apical end fails to invaginate and looks somewhat like an "apical plate." It has, in fact, been so interpreted (see Heath, 1906).

d) Sometimes the thickened basal area becomes much broader than normal and has a thinned-out region in the middle, as though a sort of fission of the vegetal pole had occurred. Such blastulae produce true identical twin

gastrulae with equivalent and symmetrical archentera. If the fission is somewhat unilateral or asymmetrical, as it often is, twin archentera of different sizes occur and the larger often becomes dominant over the smaller, determines the axis of the embryo, and absorbs the smaller.

TWINNING A RESULT OF A LOSS OF POLARITY

Physiologically considered, what happens in all these cases is this: A lowering of the developmental or metabolic rate of the embryo, either before or during cleavage, has to a more or less complete extent obliterated the original polarity, which has been shown to depend on a gradient of oxidative and other activities running from the animal or apical pole to the vegetal or basal pole of the egg or embryo. The rate of metabolism of the whole is lowered to such an extent that in extreme cases the whole gradient is obliterated, with the result that no point is distinctly apical to any other; so that any point may acquire independence and start to invaginate if stimulated so to do. If the original gradient is only partially lost certain secondary basal regions may become isolated and begin independent invagination. If the original basal region merely undergoes fission we get twin or equivalent archentera.

I look upon the process of normal gastrulation as a condition much like the formation of a new zoöid in a planarian. The ingrowth of the archenteron at the point most distant from the original apical end of the embryo is due to a physiological isolation of a new actively growing region that is highly susceptible to growth disturbances. It is the ingrowth of the archenteron that establishes the new axis of symmetry and,

indeed, the whole organization of the embryo. The old egg symmetry largely passes away and a new symmetry of the embryo and larva takes its place. In the echinoderms, of course, even this new antero-posterior axis and bilateral symmetry are largely done away with when the larva undergoes metamorphosis into the radially symmetrical adult. This, however, does not concern us in the present connection.

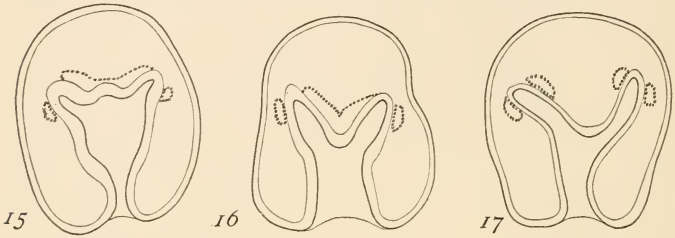
SECONDARY PHASES OF GASTRULATION

Although, as has been said, the initial steps of gastrulation involve an inpushing of a relatively basal region characterized by low metabolic rate, a new apical point soon arises at the distal or ingrowing end of the archenteron. Such a region becomes, in a sense, the head end of the larva and is the most actively growing and differentiating region of the latter. In a few cultures of *Patiria* there occurred larvae reminding one strongly of common types of double-headed human and fish monsters known technically as anadidymi, individuals divided anteriorly and united posteriorly.

ANADIDYMI AMONG STARFISH LARVAE

These larvae occurred in cultures that had been normally fertilized and in which the great majority of individuals were quite typical. What had been the cause of their occurrence was not clear. These larvae, however, were obviously retarded forms, for they were considerably smaller and less active than normal larvae. They seem to have developed normally until they had reached a late gastrula stage, a stage when it may be said that the axis of symmetry had been well established. Then, through a dichotomy of the anterior end of the

archenteron, they became distinctly "two-headed." In some of the individuals (Figs. 15, 16) the median parts were quite incompletely separated and inner structures remained united in the middle. In others, as in Figure 17, the two "heads" became separate and only the posterior part of the archenteron remained in com-



FIGS. 15–17.—*Patiria* larvae in which the anterior end of the archenteron has undergone dichotomy. Fig. 15, a larva with only a slight degree of dichotomy. Fig. 16, a larva with a moderate degree of dichotomy. Fig. 17, a larva with complete dichotomy of the anterior end of the archenteron. (From Newman.)

mon. For some unknown reason, larvae of this sort fail to advance much farther than the stages shown, and I was unable to discover the further consequences of such a process of twinning.

SECONDARY FUSIONS AND THEIR CONSEQUENCES

As has already been said, fusions frequently occur between archentera that arise closely adjacent to each other. I have rarely seen a case of fusion between two archentera that had arisen from distinctly separate basal areas. Only when the two archentera are the result of the fission of a single basal area do they exhibit a strong tendency to fuse. If the two are of equal size, i.e., are identical twins, they often grow together and fuse

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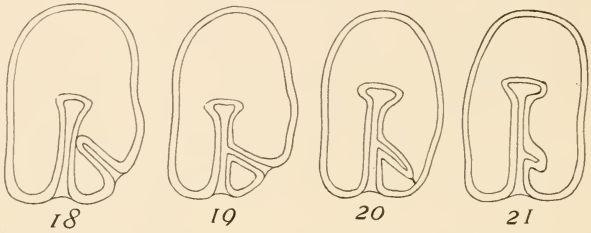
by the anterior ends of their archentera, making a type of embryo with one anterior end and a divided posterior like the type of double monster known technically as katadidymus (Fig. 5).

THE INFLUENCES OF ONE TWIN UPON ANOTHER

One of the most significant features of twinning in the starfish has to do with the apparent control one twin component has over another. When, as in cases such as those shown in Figures 1 and 2, one of the components is distinctly the primary individual and the other is secondary, only the primary archenteron is able to differentiate in normal fashion. There are numerous instances in which the primary archenteron produces its coelomic pouches and breaks through a mouth, while the secondary archenteron either closes the blastopore and becomes a cyst or else remains in an undifferentiated condition. In such cases it seems that the primary component must in some way exercise an inhibiting influence upon the secondary component. Just what may be the mechanism of such an inhibition we do not know for sure, but it seems highly probable that it is a phenomenon involving the exercise of dominance and subordination through the gradient. It is probable that the gradient of the primary individual, on meeting that of the secondary, tends to overwhelm the latter and reverse its direction, thus making it a subordinate part of the primary gradient. That this is more than a mere conjecture is evidenced by the fact that in a specimen like that shown in Figure 1 the direction of ciliary beat in the secondary component is at least mainly away from its own blastopore, instead of

toward it as it would be if this individual had control of its own gradient.

An even more complete domination of a weaker individual by a stronger occurs when one of a pair of archentera arising from one continuous basal area is distinctly smaller than the other. I have watched



FIGS. 18–21.—Four outline figures of a single *Patiria* larva, drawn on four successive days, to show the way in which a twinned larva not infrequently returns to an almost normal single or untwinned condition. The smaller twin archenteron becomes a sort of parasite upon the side of the larger. See text for details. (Original.)

from day to day all of the stages of such a process. The series of drawings (Figs. 18, 19, 20, 21) were made from a single individual at intervals of about twenty-four hours. It is remarkable how nearly like a single normal individual such a twin can become after absorbing its weaker brother, though neither one of them is truly primary in the sense that it represents the original individual, nor secondary in the sense that one is a bud from the other. This finding has a very definite bearing on theories of the origin of double monstrosities in the higher animals, especially in vertebrates. There is frequently a marked difference in the size and degree of normality in the two components in double monsters and it frequently happens that, as in the starfish cases

just described, the larger more or less completely absorbs or grows over the smaller. The larger component has come to be called the "autosite" and the smaller, the "parasite." The genetic relationship between the two has been discussed by Stockard, who considers the parasite component as a lateral bud derived from the autosite and kept in subordination to the latter much as a lateral plant bud is inhibited by the growth of the main growing-point. That such an explanation as this is inapplicable to the condition in the starfish need hardly be stated.

CONCLUSIONS

The reader will now have become aware that in the simple development of the starfish there appear, diagrammatically almost, practically all of the various phenomena that are associated with one-egg twinning. We have been able to observe all of the stages of development and to see many of the events of the twinning process. We know, moreover, to a considerable extent the causes of the various twinning processes observed. If we are to understand the more intricate twinning phenomena in the armadillo, in man, in the fishes, and in various other animals or even plants, it seems clear that we shall have to refer these conditions back to those of the starfish as a sort of norm, and it is my present opinion that the starfish situation throws much light on the whole problem of the physiology of twinning. I therefore scarcely need to offer an apology for giving first place in the present volume to the lowly starfish and for relegating to less prominent positions those phases of twinning that are more familiar and that are more imbued with human interest.

CHAPTER III

TWINNING IN EARTHWORMS AND THEIR ALLIES

One of the earliest studies of one-egg twinning was that of Kleinenberg (1878) on the earthworm *Lumbricus trapezoides*. The condition described by him is of great interest in our study of twinning, as it was perhaps the first described case of twinning in which it was certain that only one egg was involved.

Kleinenberg found that of the three to eight eggs found in a cocoon or capsule only one, or occasionally two or three, undergoes development, the others, which he believes to have been unfertilized, undergoing complete disintegration within the capsule. Although the author does not lay any stress upon this condition, I would like just here to point out that the environment of the developing egg or eggs in a capsule, fouled by the decay of several other eggs, is not at all likely to result in normal development.

The embryonic history of the eggs that survive is as follows: Cleavage is apparently normal up to a blastula stage, consisting of a thin-walled, bladder-like vesicle. Gastrulation apparently occurs through the more rapid growth of the cells at one end of the vesicle. At the pole of greater thickness two large cells, the primary mesoblast cells, become pushed in and are overgrown by small surface cells. From these two mesoblast cells there arise by proliferation two rows of cell masses destined normally to be the right and left endodermal and mesodermal

structures of a single animal. While this process of mesoblast elongation is taking place a longitudinal constriction occurs, beginning at about the middle and running both forward and backward until it surrounds the embryo. This furrow cuts more or less deeply through the embryo, dividing the right half from the left. In the majority of individuals the two half-bodies remain connected in the middle region by only a few large ectodermal cells. Each half-embryo, when thus isolated, develops into an entire embryo. Here we have a true case of duplicate twins derived from the right and left halves of a single embryo. When the slightly joined twin worms become active they complete their physical separation by means of a series of rotations which twist and finally break off the uniting cellular cord.

Double monsters result when the connection is too thick to admit of twisting apart. Sometimes the united region is of considerable extent, but the union involves only the external epithelium and not at all the internal structures. Sometimes the two components of these double monsters are of very unequal size, one of them being the equivalent of an autosite and the other of a parasite, if we may use the terminology employed for similar situations in vertebrate conjoined twins. One interesting feature of Kleinenberg's work is that, in the species studied by him, twinning appears to be almost as regular and specific a process as it is in the armadillos. In all of his numerous cases only a very few instances occurred in which but a single worm emerged from an egg capsule, and even these single worms were probably survivors of twins, for rudiments

of a degenerate or absorbed twin were usually distinguishable.

Vejdovsky (1882–92) a few years later made an extensive study of twinning in three other species of earthworms, *Lumbricus terrestris*, *Allolobophora foetida*, and *Allolobophora trapezoides*. Only two twinned specimens were found in the first species, two in the second, and large numbers in the third. He found a great variety of conditions which may be summarized as follows: (1) One case in which the components were united on the ventral side along the whole length of the body. (2) Cases in which union was along the dorsal side (frequent). (3) Cases in which union was end to end (rare). In all of these types, except in those where the union was throughout the entire length, there occurred cases of more or less marked size inequality between the two components. Vejdovsky states that completely separated twins occur with extreme rarity in the species studied by him.

Weber (1917) made a study of double monsters in the earthworm *Helodrilus caliginosus trapezoides*. In this species there occur many completely separate twins as well as large numbers of conjoined twins. It is also important to note that in nearly a third of the cases observed an egg gave rise to but a single individual and that a few eggs produced quadruplets. The majority formed double monsters. Since some significance attaches to the manner in which the components of these conjoined twins were united, certain details of Miss Weber's observations must be given. She classifies the double monsters according to their mode of union as follows: (1) Those in which the union is dorsal;

(*a*) those in which the union extends to the alimentary tracts; (*b*) those in which alimentary tracts are not united. (2) Those in which the union is latero-dorsal (one example). (3) Those in which union is end to end and in which the cerebral ganglia are double and on opposite sides of the digestive tract. The union is dorsal, as the nerve cords are on the free (opposite) sides of the united region. (4) Those in which union is lateral, the components lying side by side with the mouth of both on the same side. (5) Those in which the two components are extremely unequal in size.

It is a significant fact that no cases were found in which the union is ventral. This leads Miss Weber to believe that the single instance cited by Vejdovsky is a misinterpretation and that what he had was a case of dorsal union, the confusion being due to the close approximation of ganglia from opposite sides which lay in the semblance of a paired arrangement.

As to the causes of twinning in the earthworms Kleinenberg makes the unsupported suggestion that the doubling is due to the entrance of two sperms into a single egg. Such a suggestion is nowadays quite untenable. Vejdovsky is inclined to adopt a physiological explanation suggesting the possibility that twinning may be due to environmental factors, such as temperature, moisture, or exposure to air.

Korchelt (1904) described and figured (Fig. 22) an interesting double monster of the earthworm *Allobophora subrubicunda*. This is a very typical case and will serve as a type for the whole group. This author also succeeded in producing large numbers of double-headed and double-tailed worms by regeneration methods. After

cutting out a section of worm from near the middle, the anterior and posterior ends regenerate very frequently into double or even triple heads or tails. Doubling or twinning in the course of regeneration after cutting the body or appendages of animals or plants is an extremely common phenomenon. I consider that the slowness with which early regrowth occurs allows the axiate organization of the tissue to be lost, and new growing regions arise sometimes at two or more places, thus resulting in duplicity or triplicity.

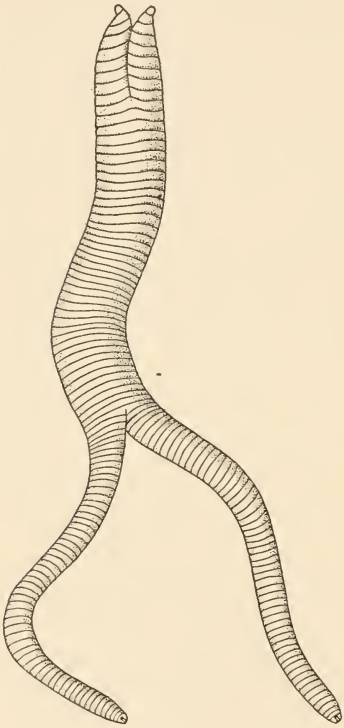


FIG. 22.—Typical twin earthworm of the species *Allolobophora subrubicunda*, slightly bifid at the anterior end and extensively bifid at the posterior end. In other larvae the condition may be reversed or else one end may be single and the other bifid. (After Korschelt.)

TWINNING IN THE MICRODRILOUS OLIGOCHAETES

The very recent account of Welch (1921), although not illustrated, affords data of very considerable significance with reference to the phenomenon which he calls bifurcation. He made a study of the contents of over 500 cocoons of *Tubifex tubifex*, which were obtained

from pond and river mud by means of a sieve. Each cocoon contains from one to fourteen eggs or embryos, the average being about nine. The only avenue of escape from the cocoon is through the two necks of the latter. When temporary plugs in these necks are removed the apertures are just wide enough for a normal young worm to emerge.

About 20 per cent of the cocoons examined showed one or more double-headed or double-tailed worms, only one, two, or three bifid worms being present in each. The various types of duplicity are classified by Welch as follows:

A. Either anterior or posterior extremity bifid
  I. Bifurcation simple
    a) Branches equal
    b) Branches unequal
  II. Bifurcation compound
    a) Plane of bifurcation
      (1) Secondary bifurcation in same plane as primary
      (2) Secondary bifurcation at right angles to primary
    b) Equality of bifurcation
      (1) Parts of primary bifurcation equal; secondary equal or unequal
      (2) Parts of primary bifurcation unequal; secondary equal or unequal

B. Both anterior and posterior extremities bifid
  I. Primary bifurcations in same plane
  II. Primary bifurcations in different planes
  III. Either or both bifurcations compound

It was further brought out that anterior bifurcations with normal posterior end are about twice as numerous as those with posterior bifurcation and normal anterior end. Furthermore those with both ends bifurcated

are about as numerous as those bifid at the anterior end alone.

It is very rare for bifid individuals to be able to emerge from the cocoon, only ten young worms out of 4,000 that had emerged being bifid. The forked ends tend to inhibit emergence because the opening of the cocoon is only large enough for a normal worm. Those with deep bifurcations both anteriorly and posteriorly could not possibly escape. The only chance of successful emergence seems to be to start out with the single end first. A few escaped that had a deep bifurcation at one end and a very slight one at the other. Thus the hazards of one-egg twinning seem to be even greater for worms than will be shown to be the case in man. If there were in these worms an inherited tendency to twin, it could not successfully be passed on, for the survivors are far too infrequent to admit of such a character being transmitted in so large a percentage of individuals as actually occur in cocoons. The only alternative conclusion, then, is that the condition is due to environmental factors such as those already discussed.

THE MODES OF TWINNING IN THE OLIGOCHAETES

The work of Hyman and others has shown that in annelids, and especially in oligochaetes, the head end is at first the only apical end and it is highly susceptible to growth-inhibiting agents. Relatively early in development, however, the posterior end of the embryo becomes a secondary apical point with a forward-directed gradient. Thus these worms have a double axiate organization with two highly susceptible regions. It is very significant that bilateral doubling (true twinning) occurs

most frequently and is most complete in the two apical regions of the double gradient and that the part of the body that most frequently fails to undergo twinning is that region which represents the common basal region, or region of lowest metabolic rate, of the two gradients.

It is also important for us to note that such bilateral organisms as the worms show almost exactly the same types of double monstrosities as have been described for the vertebrates. The explanation of the fact that heads and tails are double and the middle of the body single is that the free ends have undergone bifurcation or longitudinal fission. There is not even the shadow of suggestion that the double monsters arose as products of the fusion of two individuals arising from separate embryonic axes, for there is really no possibility that such a thing could occur in these worms. The fact that all sorts of double monsters, in these simple, bilateral, metameric organisms actually do occur by dichotomy of the apical ends goes far to support our theory of the origin or conjoined twins in the vertebrates. It should also be said that every degree of bifurcation is present, from a very slight terminal broadening to a very deep bifurcation running nearly half the length of the worm. This situation also entirely parallels the condition seen in the vertebrates and shows that individuals vary greatly in their responses to the factors that induce twinning. Another situation in these double worms that parallels that in vertebrate double monsters is that the portion of the body that is apparently single is in reality nearly all double. Thus there are usually two complete nerve cords in the united part of the body and they are 180° apart; which means that the ventral

parts are separate and the dorsal united. This is just the opposite of what we find in the vertebrates, but is precisely what we would expect in view of the fact that the nervous system is dorsal in the vertebrates and ventral in the annelids.

CAUSES OF TWINNING IN THE OLIGOCHAETA

The method of reproduction in the Oligochaeta is the well-known one of cocoon formation which, at the risk of repeating what every biologist knows, may be stated briefly as follows: During copulation a tough girdle composed of hardened slime is formed about the clitellum of each worm. After the pair separates, each slime girdle which is destined to be a cocoon is slowly worked forward, collecting albumen from the glands on the ventral surface. It is sloughed off over the head, passing first the openings of the oviducts where it receives eggs, then that of the seminal receptacles where it receives sperm. The two free ends close as though with a drawstring and the closed cocoon is dropped on the ground or in the mud. It has already been pointed out that, at least in some species, some of the eggs die and decay. This would greatly foul the contents of the cocoon. Whether some eggs die or not, the living eggs must develop with a limited supply of oxygen. It is this condition, I believe, that is at the bottom of the twinning process. Lack of oxygen probably so retards early development at a time when abundant oxygen is demanded that the bilateral primordia become physiologically more or less completely isolated. Isolation occurs more extensively at the points of highest rate of metabolism, the anterior and posterior

ends and the ventral surface, and is less complete at regions of lower rate of metabolism, the middle parts of the body and the dorsal side. In other words, inhibition strikes the apical points of the axis of polarity and the axis of symmetry, just as our general theory demands.

The type of twinning that prevails among the annelids is bilateral twinning or isolation of the right and left side of the single embryonic axis. This type of twinning was only occasionally met with in the echinoderms, where the prevailing mode is one which involves double or triple gastrulation, or the formation of paired embryonic axes. Since both of these modes of twinning are common throughout the animal kingdom, it seems well to have shown them in unequivocal form in groups where the processes are clear and the causes known at least to a high degree of probability. It is my belief that the analysis of the double-monster situation in the earthworms practically settles the controversy as to the mode of origin of vertebrate conjoined twins, and this chapter is therefore placed well toward the beginning of the book because of its bearing on the equivalent conditions in the vertebrates about which there have been widely divergent opinions.

CHAPTER IV

ONE-EGG TWINS IN FISHES

INTRODUCTION

The existence of one-egg twins and double monsters in fishes has been known for a long time. Possibly the earliest reference in the literature to this subject is that of Aldrovandus (1642) in his *Monstrorum Historia.* Since then probably no less than a hundred papers and monographs, describing one or more specimens of fish exhibiting some degree of duplicity, have been published. Most of these authors have apparently been under the impression that they were reporting some new and strange monstrosity and their accounts have been superficial. A number of them have secured considerable collections of eggs containing twins or double monsters, and practically the same series of monstrous types has been shown to appear in all representative collections.

As to the frequency with which twinning occurs in fishes we have only a small amount of evidence. Rauber found two double monsters among 1,000 trout eggs; and one in 325 pike eggs; Coste found over 100 double monsters in about 400,000 eggs of various species; Lereboullet found 222 double monsters in 203,962 eggs of the pike. The percentage of twins is so small that the collection of an adequate series of specimens must be a task of some moment. Among the kinds of fishes in which double monsters have been described we may mention the following: sharks, skates and rays, lung-

fishes, salmon, trout, mackerel, perch, pike, and killifishes. The trout is by all odds the favorite type and no less than twenty-five separate reports of duplicity in trout eggs have appeared. Some significance attaches to the fact that the trout shows a higher incidence of duplicity than other species. In the first place the trout is the favorite game fish of the world and is more commonly reared artificially in hatcheries than is any other fish. This alone would account for the more frequent observation of monstrosities of various sorts, but would not account for the higher percentage of double monsters and twins. It seems probable that the trout, being a fish of the cold, pure, and thoroughly oxygenated waters of streams and spring-fed lakes, is more abnormally environed during development in crowded hatcheries than would be most other fishes. The key to the cause of twinning doubtless lies in this circumstance, as we shall later attempt to show.

Of the various authors who have contributed to our knowledge of twinning in fishes the following, placed in their chronological order, seem to me to deserve especial attention: Lereboullet (1855 and 1861), Knoch (1873), Rauber (1877, 1878, and 1879), Klausner (1890), Windle (1895), Kopsch (1899), Schmitt (1901 and 1902), Gemmill (1901 and 1912), Stockard (1921).

MODES OF ORIGIN OF FISH TWINS

Various classifications of fish twins have been given by different authors. That of Gemmill (1912) seems the most satisfactory of those hitherto published.

Whatever be the causation, we may recognize in vertebrates generally, four somewhat different *modes of origin*, whether for

double (and multiple) monstrosities, or for double (and multiple) unioval separate embryos.

These different modes are: (1) The appearance of two (or more) embryonic rudiments on a single blastoderm. (2) The presence in the egg of two (or more) separate blastoderms. (3) Fission or dichotomy on the part of a single embryonic rudiment. (4) Formation of certain axial structures in two parallel sets on a single embryonic rudiment.

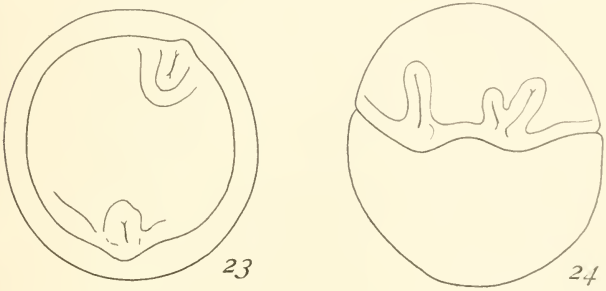
Modes 1 and 2 are doubtless exactly equivalent to the similar modes described in chapter i for the starfish. Modes 3 and 4 are, I believe, merely different degrees of the same process of longitudinal fission, which involves the more or less complete bilateral separation of the two sides of the bilateral blastoderm.

Gemmill considers that the first mode is universal for fishes, that the second is represented, in fishes, only by a single instance in the literature (Klausner's case cited below), and that the third and fourth modes of origin are extremely rare in fishes.

There is little doubt, I believe, that separate one-egg twins may and do originate by means of both of the first two modes. Several writers have described instances of germ-ring stages in which there were two or more embryonic shields. Rauber, especially, has given us unequivocal examples of this mode of origin as shown in Figures 23 and 24. Such examples as these are evidently instances quite completely equivalent to the commonest type of twinning in the starfish, involving a loss of axiation of the single blastoderm and the production of two or more regions of gastrulation instead of the original one. Rauber's second figure (Fig. 24) shows on the right side what I believe is an early stage of a double monster resulting from the dichotomy of an origi-

nally single embryonic shield. It is not, as Gemmill believes, the product of the fusion of two adjacent embryonic shields.

Twins originating from plural invaginations of the margin of the germ ring must inevitably come to lie parallel to each other with heads pointing in the same



FIGS. 23, 24.—Germ-ring stages of twin trout embryos. Fig. 23, an embryo with two equivalent embryonic shields, destined to form separate twins. Fig. 24, an embryo with two embryonic shields, one destined to form a single separate individual and the other a two-headed double monster. (After Rauber.)

direction. They must also have a common yolk sac and yolk stalk. Such twins could therefore never become fully separate.

The question arises as to whether it would be possible for fish twins to arise in such a way that they would be separate when hatched. Klaussner (1890) cites one case of fish twins which might possibly have become separate after hatching. This pair of twins was found lying side by side on one egg but with heads pointed in opposite directions. Obviously such a condition could not have arisen as product of the invagination of two

points upon one germ ring. They therefore must have come from two separate blastoderms. The two blastoderms probably arose through the physiological isolation of the first two blastomeres, as so frequently happens in parthenogenetic *Patiria* eggs. This mode of origin of fish twins must be extremely rare, for no other such cases are on record.

Gemmill is of the opinion that the third mode of twinning is found in fishes only in connection with "the peculiar and imperfect doubling characteristic of the hemididymous condition." Two phases of hemididymus are distinguished: (*a*) mesodidymus, in which there is apparent doubling of the middle region while the anterior and posterior ends remain single; (*b*) katadidymus, in which the anterior ends remain single and the posterior ends are double.

As compared with anadidymus, in which the anterior end is double while the posterior end is single, the two forms of hemididymus are extremely uncommon, and are rarely if ever found in advanced embryos. They seem to be due to a mechanical pulling apart of the bilateral primordia during germ-ring overgrowth. Kopsch was able to get katadidymus in trout eggs by injuring the blastoderm at the posterior end of the embryo. Knoch found instances of katadidymus in cases where eggs were rather roughly handled by violent stirring. In general the condition is more like *spina bifida* than true twinning and may, I believe, be dismissed without further consideration. Gemmill's fourth mode of twinning is illustrated for the fishes by a single example of a salmon embryo, reported by Barbieri (1906), in which there is a marked tendency for ventral organs to show greater

duplicity than dorsal ones. Gemmill interprets this condition as the result of the origin of two axes in a single embryonic shield accompanied by secondary fusion of dorsal structures.

It should be emphasized that the last three modes of twinning, as interpreted by Gemmill, are extremely rare among fishes and that none of them have been noted by those observers who have made large and representative collections. Cases of hemididymus have been observed mainly in quite early germ-ring stages and there is ground for believing that separations observed and figured are merely cases of mechanical dehiscence more or less temporary in character. The case of Barbieri, cited above, is so extraordinary and so unlikely that, until confirmatory instances have been described, it seems hardly worth while to speculate about its significance.

If we put aside the rare, exceptional, and poorly understood types of duplicity in fishes we find that there remain only two definite types of twinning:

a) *Separate twins*, in which the entire bodies of the two individuals are separate with the exception of unions in the yolk-sac region. On account of mechanical limitations it does not appear possible for fish twins arising from a single blastoderm to be entirely separate. Morphologically speaking, however, such individuals are equivalent to separate one-egg twins in mammals and will be considered here as separate twins.

b) *Conjoined twins of the anadidymus type*, in which there is anterior duplicity for a greater or less distance and posterior singleness. This is the only type of fish double monster described by those who have made large collections.

These two standard classes of fish twins will now be considered in detail.

SEPARATE TWINS

MODE OF ORIGIN

All of the writers who have attempted to formulate theories of the mode of origin of fish twins in general have offered the same explanations for both separate and conjoined twins. Gemmill's view is representative of all such views and is expressed as follows:

> The recorded observations indicate that double-monster fishes (including those united by yolk sac only) always arise on a single yolk and from a single blastoderm at the margin of which two more or less separate centers of gastrulation and embryo-formation have appeared.
>
> The twin centers of embryo-formation mentioned above may be classed in two groups (*a*) and (*b*), according to the distance which separates them from one another. (*a*) In the first and most important group the interval is not too great to prevent approximation and union of the two embryonic axes from taking place during the natural course of their growth in length. . . . . (*b*) In the second group, the twin centers of embryo-formation are so far apart that there is no compelling influence of the kind described above which would lead to the approximation and union of their growing embryonic axes. Accordingly the twin bodies remain separate, except for the adventitious union supplied by the layers forming the common yolk sac.

I see no reason to doubt that at least many of the truly separate fish twins arise in the manner described above. But I have reason to believe that at least some separate twins and all truly conjoined twins arise by partial dichotomy or by complete fission of the right- and left-hand primordia of a single axis. In other words, there may be two types of separate twins, those that

originate from separate embryonic axes or centers of gastrulation and those that originate from the fission of a single embryonic axis. As will be shown below, all true anadidymi must be viewed as incomplete fission products of a single axis. If this view be true, some separate twins may well belong to the same series as the anadidymi, but not in the sense of Gemmill, who believes that all arise as separate centers of gastrulation and that conjoined twins are products of fusion.

ORIGIN OF AUTOSITE AND PARASITE TWINS

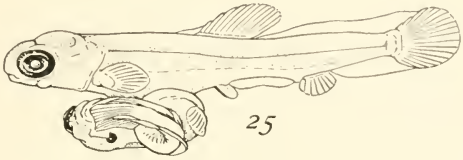
It is just here that we may profitably turn back to the conditions described for the starfish. It will be remembered that very frequently the original axis of the blastula is only partially obliterated so that the main basal area, or region of gastrulation, persists while one or more minor basal areas may have been established. Such minor areas give rise to secondary archentera, smaller in size and situated at regions where archentera would not be expected, sometimes appearing at the opposite pole or apical end of the blastula. In the starfish these secondary archentera persist for a time but, in most cases, are completely inhibited and subsequently become pinched off and absorbed. In a few cases the secondary archenteron may be nearly as large and active as the primary, and may persist as long as the larva lives. Now in the fishes something very much like this probably takes place. When the inhibition has been insufficient entirely to obliterate the original axiate relations of the blastoderm, the original gastrulation area persists; but a secondary area arises probably at the opposite side of the blastoderm. This secondary area, after beginning

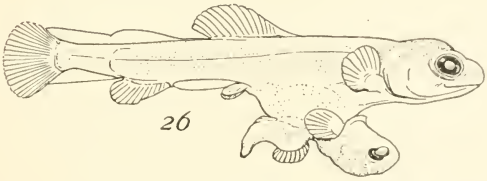
to invaginate and to form an axis, may become inhibited through the much more active growth of the primary axis and may become obliterated. Stockard, for example, noted a number of cases in which secondary embryonic shields appeared but no accessory embryos were formed. If, however, the secondary embryonic shield is allowed to develop far enough it cannot be completely suppressed by the primary embryo and a secondary or subordinate embryo will develop. Such an embryo will always, I believe, exhibit some evidences of being inhibited, such as small size, cyclopia, and other defects. The primary embryo, as in the starfish larvae, may grow quite normally, apparently suffering no detriment from the presence of the secondary embryo, until they have mutually surrounded the yolk sac and thus come to lie belly to belly, attached by means of the vitelline tissues. When this happens the larger primary embryo tends to grow around and absorb the smaller secondary embryo and frequently succeeds almost completely in doing so. The larger larva is here the autosite and the smaller one the parasite (Figs. 25 and 26). It is my opinion that the condition of autositism and parasitism in fishes practically always arises in this fashion. Cases of false autosites and parasites have been described by Stockard in connection with true conjoined twins derived by the separation of parts of a single embryonic axis. This condition does not concern us here, for we are dealing with twins derived by plural gastrulation.

ORIGIN OF TRUE DUPLICATE TWINS

Harking back once more to the starfish situation, it may be recalled that a very common type of twin

larva was one in which the original polarity of the blastula had been largely obliterated and in which two new twin gastrulation areas had arisen, each of which is a secondary area and each quite definitely a mirror-image duplicate of the other. Neither one is primary to the other and neither one tends to inhibit the development of the other.



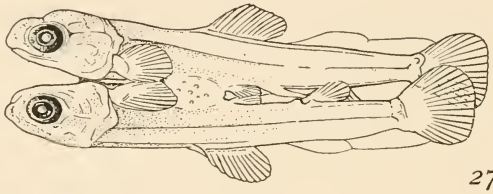


FIGS. 25, 26.—Typical examples of trout twins of the "autosite-parasite" variety. In both cases the autosite is nearly normal and the parasite decidedly subnormal. (After Stockard.)

They grow at equal rates and form identical twin axes. The condition may be said to be due to the physiological isolation of the two halves of the original blastoderm.

Now just such a process as this takes place, I believe, in the fish blastoderm and accounts for the numerous cases of duplicate twins attached only by the yolk sac or by parts of the lateral body wall. The two individuals are each complete and normal and of approximately equal size. Examples of such duplicate twins are seen in Figures 27 and 28. The differences with regard to the

relative positions of these twins and their points of attachment depend, I believe, upon how far apart on the blastoderm the twin gastrulation areas occur. It may be recalled that, in the duplicate twin starfish larvae, the archentera sometimes occurred closely side by side, and sometimes they occurred as far apart as possible.



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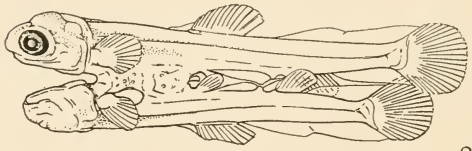

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FIGS. 27, 28.—Typical "separate" trout twins arising from double symmetrical gastrulation. Fig. 27, a pair of identical twins, both normal. Fig. 28, a pair of such twins, one of which is somewhat smaller and defective in the head region. (After Stockard.)

Doubtless the same is true for the fishes. If the two individuals arise close together we would expect them to be united by their inner sides; but if they arise on opposite sides of the blastoderm, they should be facing one another belly to belly. And there would be many intermediate stages. Such unions never involve any more than mere external fusions, axial elements such as notochord, neural tube, and alimentary tract never being united.

When two individuals have arisen from two closely approximated embryonic axes they may be crowded together laterally so closely that the structures of the inner sides, such as the pectoral and pelvic fins, may come to be more or less fused and crowded out of place; but this situation is only to be expected as the result of such close quarters. This kind of fusion, however, is quite different in principle from the sort of primary fusion that Gemmill and others invoke in order to account for double monsters.

CAUSE OF SUBNORMAL DEVELOPMENT OF ONE TWIN

When two embryos arise by plural gastrulation one of the individuals is often subnormal throughout its life. Is its subnormal condition due to an inhibition exerted by the larger embryo, or is it the result of being subordinate from the time of its origin? My theory is that in many cases the original axiate relations have persisted to some extent; that on this account any individual arising from a secondary gastrulation area was from the first subordinate and inhibited owing to its origin from a region that still belonged, at least partially, to the original axis of the single individual. In extreme cases such a secondary axis is rather promptly suppressed when the primary individual regains its normal growth momentum. In less extreme cases the secondary individual is able to maintain some degree of independence, but is handicapped at first by its slower start and secondarily by its contact with the larger, more vigorous individual.

In so far as the mode of origin of separate fish twins is concerned, with the possible exception of those with

situs inversus viscerum, there is no disagreement between Gemmill and his followers and myself, but I cannot accept their theory of the origin of conjoined twins, which are really much commoner in fishes than are separate twins and have excited much more comment and interest than the latter. The next chapter is to be devoted exclusively to conjoined twins or true double monsters, their causes, and mode of origin.

CHAPTER V

DOUBLE MONSTERS OR CONJOINED TWINS IN FISHES

CLASSIFICATION AND ANATOMICAL STRUCTURE OF CONJOINED TWINS

The earliest adequate classification of conjoined fish twins is that of Windle (1895), who concerns himself only with the external evidences of duplicity. He recognizes eleven classes of trout twins characterized by the following structural peculiarities:

1. Three eyes of the same size (the least manifestation of duplicity noted by any of the authors).

2. Three eyes, the median being larger than either of the lateral ones.

3. Four equal-sized eyes.

4. Two heads, the duplicity extending as far back as the otic region.

5. Duplicity extends to the region of the pectoral fins.

6. Duplicity extends to the posterior border of the yolk sac, the caudal extremity of the fishes being quite single.

7. Duplicity extends a short distance behind the posterior border of the yolk sac, but the caudal extremity is quite single.

8. Duplicity extends to the posterior border of the yolk sac. Behind this there are two caudal extremities overlapping one the other and firmly united by their contiguous aspects.

9. Union by caudal extremities alone (not seen by Windle himself, but noted by early writers).

10. Union by ventral aspects at the site of the attachment of the yolk sac.

11. Parasites. (All cases in which one individual is distinctly smaller than the other and strongly united to it.)

Windle's classification, though dealing only with superficial characteristics, emphasized, rightly I believe, the degree of duplicity rather than that of union in all cases of incompletely double individuals. His first seven classes are, in my opinion, true instances of anadidymi or products of incomplete fission of a single bilateral primordium. The remaining four classes represent twins derived from separate embryonic axes, but more or less secondarily fused by external parts. These, according to our theory, are separate twins and do not logically fall into the category of double monsters. Windle has no theory of the origin of these forms to advocate, his paper being purely descriptive.

CLASSIFICATION OF GEMMILL (1891)

In his early paper, a summary read before the Royal Society of London over thirty years ago, Gemmill gave us a very painstaking classification of a large collection of trout twins and double monsters, based on the study of the internal relations of the connected individuals. It will be noted that he emphasizes union rather than duplicity. His classification is as follows:

Type 1. Union in head region:

a) The twin brains united at the mesencephalon.

b) The twin brains united at the medulla oblongata.

Type 2. Union in pectoral region:
- *a*) The pectoral fins absent on adjacent sides.
- *b*) The pectoral fins present, but united on adjacent sides.

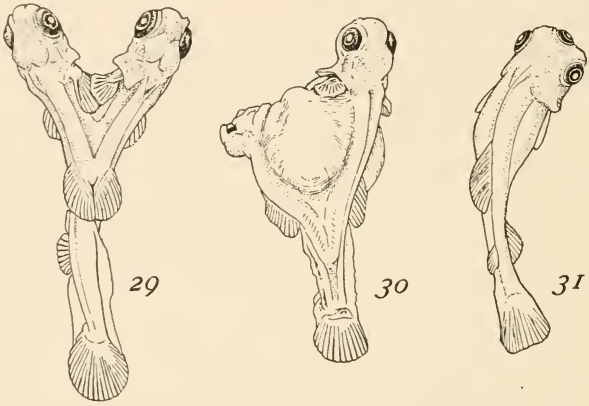
Type 3. Union behind the pectoral region:
- *a*) The twin bodies united at a considerable distance in front of the vent.
- *b*) The twin bodies united close to the vent.

Type 4. Union by yolk sac only.

The first three types should be classed as conjoined twins or double monsters and the fourth type as separate or duplicate twins. The series is evidently a very complete one in which every degree of duplicity of the primary axis, from a slight twinning in the most anterior region to complete separation, is found. There are no cases of double tails unless the twins are entirely separate.

By far the commonest type of double monster is that in which the process of dichotomy extends well into the abdominal region, but not past the pelvic region. Such an individual is shown in Figure 29 (p. 54). As a rule individuals showing this degree of duplicity show approximately equal development in the two anterior components. When the duplicity extends farther toward the posterior end, as in Figure 30, there is a greater tendency for one component to be abnormal. Cases in which the duplicity is confined to the most anterior structures are relatively infrequent. Such a type is shown in Figure 31, in which the duplicity is confined to the forebrain, and there is but one median eye, belonging partly to one and partly to the other incompletely double head. There are probably cases of twinning involving even less than this, but no good figures of such were to be found.

Although it seems to me that Gemmill's data almost automatically speak forth the fission theory of twinning, it is perfectly obvious that throughout he has in mind exactly the opposite process. He always speaks of the uniting of paired parts or regions into single elements. All of his types of twins are interpreted as cases of more



FIGS. 29–31.—Typical examples of trout double monsters, arising from the partial fission or dichotomy of a single embryonic axis. Fig. 29, the commonest type of trout twin, exhibiting *situs inversus viscerum*. Fig. 30, a case in which the fission products are unequal, one being decidedly subnormal. Fig. 31, a rather rare type of minimal twinning, the dichotomy being confined to the most anterior structures, the eyes and the forebrain. A horizontal section through such a type is shown in Fig. 57. (Figs. 29 and 30 after Stockard, Fig. 31 after Gemmill.)

or less complete fusion of two individuals. He implies that conjoined twins are derived from originally separate individuals that have been brought together by the mechanism of germ-ring closure and have fused more or less completely. This idea naturally leads to a far-reaching conception of the mode of formation of bilater-

ally symmetrical organisms: the now largely discredited concrescence theory.

In his more general work on the teratology of fishes Gemmill (1872) adheres to his original idea of double monsters as products of partial fusion of originally separate embryos and accounts for the degrees of separateness of the anterior parts in an ingenious way. He considers the germ ring "as a stock, able to give rise vegetatively, so to speak, to more than one embryo." As a rule only one embryonic shield arises on the germ ring, but the germ ring is believed to be potentially capable of giving rise to accessory embryonic shields at any distance from the first. When in this way two shields arise, the level of the point of union "varies directly with the original distance between the two centers of embryo formation."

In view of the fact that the validity or non-validity of this "budding theory" of twinning in fishes depends on a proper interpretation of the nature of the germ ring and the mode of formation of the embryonic axis, the reader will doubtless be indulgent enough to allow us to present a summary of the evidence on this point.

THE CONCRESCENCE THEORY AND THE INTERPRETATION OF CONJOINED TWINS IN FISHES

According to the concrescence theory the embryonic shield represents merely the head end of the future embryo, while the lateral halves of the rest of the body are separated from each other and are represented in the germ ring. As the germ ring passes the equator of the yolk it concresces to form the bilateral elements of the embryo. This is evidently the view originally

taken by Gemmill, and if true would apparently account for the partial uniting of two embryonic axes into one in the case of conjoined twins. Since the germ ring is supposed to represent the two sides of the axis, what would happen if a second head or embryonic shield arose to the right or to the left of the original shield? Obviously the two heads would be in competition for that region of the germ ring between them. The germ ring is gradually taken up partly by one embryo and partly by the other. They would therefore have separate inner sides as long as the common region of the germ ring lasted, but they would sooner or later use this up and neither embryo would have any more material for its inner half. Beyond this point, therefore, the two outer halves of the germ ring, one half belonging to one embryo and the other to the other, would concresce in the median line to form the single or untwinned part of the body. Thus we would readily obtain embryos with two anterior regions and a single or common posterior region. The farther apart the embryonic shields arise on the germ ring the greater the extent of the twinned or double region. If embryonic shields arise on opposite sides of the germ ring the twins would be entirely separate, except that they have a common yolk stalk, a condition that could hardly be avoided since the two embryos must have a common blastopore and are on a single yolk sac. This all sounds reasonable enough on the basis of the concrescence theory, but unfortunately this theory is now practically discredited. No longer can it be maintained that the germ ring represents the separated right and left halves of the embryonic axis, for it has been proved experimentally by several reliable

workers that the germ ring does not concresce to form the embryonic axis.

KOPSCH'S VIEW OF EMBRYO FORMATION

In a very able monograph entitled *Untersuchungen über Gastrulation und Embryo-bildungen bei den Chordaten*, Kopsch (1904) has brought out the following facts:

a) If with a hot needle a portion of the germ ring near an early embryonic shield is killed, such injury has no effect upon the organization of the future embryo but merely results in the formation of a scar in the tissue outside of the embryo proper. This was true no matter how close to the embryonic shield the injury was made.

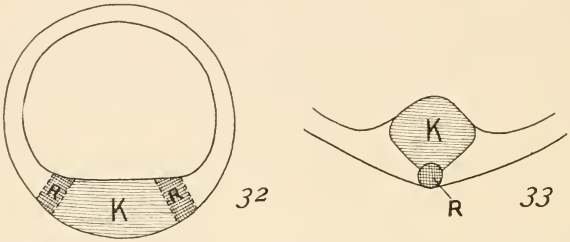
b) If the posterior end of the embryonic shield is injured, the entire embryonic body is formed except the tail and adjacent tissues. This means that the whole body is represented in the original embryonic shield and is not built up by germ-ring concrescence.

c) The embryonic shield, as soon as it is definitely established, contains the complete embryonic axis from head to tail. At first the tail proper is represented only by a tail-bud, which begins to grow out even before the germ ring has completed its envelopment of the yolk.

d) The only evidence of any concrescence at all is seen in connection with the formation of the *Knopf* or tail-bud region. At first this region appears to arise at the two outer margins of the thickened portion destined to form the embryonic shield. These bilateral regions appear to grow in, apparently more by migration of cells than by gross concrescence, to form a median posterior area of the shield which is essentially the tail-bud.

e) As soon as this *Knopf* region is organized the axis of the embryo is complete and all increase in the length of the axis occurs in a growing zone between the head and the tail-bud.

That this is not merely one man's interpretation of these conditions is seen in the fact that essentially the



FIGS. 32, 33.—Diagrams showing Kopsch's interpretation of the mode of embryo formation in the teleost fishes. *K*, the *Kopf; R*, the *Knopf*. For explanation see text. (From Kopsch.)

same position is taken by Morgan, Virchow, Sumner, Jablonowski, and other workers on teleost embryology. Professor F. R. Lillie, who has given much attention to this matter, considers the position of Kopsch entirely sound. I propose then to accept this consensus of judgment about the nature of concrescence and the formation of the embryo in teleosts. Kopsch's conclusions on these problems are as follows.

When the embryonic primordium is still merely a somewhat thicker sector than the germ ring (Fig. 32), there is a middle region (*K*) the *Kopf* (or head) and two laterally arranged areas (*R*), the *Knopf* (or tail-bud). When the embryonic shield proper is formed there appears to be a sort of migration forward and toward the median line of the cells of the *Kopf* region, which is

possibly to be interpreted as concrescence. Similarly the *Knopf* regions come together to form a single median tail-bud, which completes the embryonic axis and is to be considered as the final step in this very limited process of concrescence (Fig. 33). Once the germinal shield is formed, the axis is to be considered as completed, and no farther concrescence is possible except a slight migration inward of some of the adjoining regions of the germ ring to form lateral regions of the embryo. There is no opportunity for a median coalescing of outlying regions of the germ ring to form any part of the embryonic axis.

If this view is valid, it has a most important bearing on our interpretation of conjoined twins in the fishes as well as in other vertebrates. Such double-headed and single-bodied individuals could not possibly arise from a fusion of two separate embryonic shields, as Gemmill's budding theory implies. In order to make such a fusion possible it would be necessary to suppose that in some extraordinary way the inner halves of each twin becomes obliterated during the fusion process so that only the outer halves remain, and that these outer halves come together in such an extremely precise way as to fuse notochord with notochord, neural tube with neural tube, aorta with aorta, vein with vein, intestine with intestine. We also would have to suppose that at the time of fusion the two components were in exactly the same stage of development, for otherwise the parts that come together in the median line would not represent equivalent regions in the primary axis, and there could be no exact equivalence of contribution to the united posterior part of the body.

In his later work Gemmill (1912) accepts the interpretation of Kopsch as to the formation of the embryonic

axis and the nature of the germ ring, but persists in the idea that double monsters are the result of a fusion of originally separate embryonic axes. His own statement makes this clear:

> Approximation and union (of the twin centers of embryo-formation) are due to the factors to which attention was directed above, namely, the utilization during growth of the blastodermic margins near the primitive streak, and the slowness of expansion on the part of the blastoderm over the yolk in this same region The twin adjacent axes are inevitably brought together posteriorly through the disappearance of the interval between them. The process may be called one of *primary fusion*, in contrast with a process which often supervenes later, and which consists in the *secondary fusion* of organs or structures already laid down.
>
> Primary fusion takes place earlier or later, i.e., in the head-, body-, or tail-region, according to the interval which separated the embryonic rudiments when they first appeared. In other words, the degree of duplicity varies directly with the original distance between the two centres of embryo-formation. When the union is a purely lateral or an approximately lateral one, the posterior united part finally becomes simply and perfectly bilateral. This takes place through the gradual fusion and disappearance of structures belonging to the left and right halves respectively of the right and left component embryos. Thereafter the right and left halves of the right and left embryos unite naturally to form a normal bilateral body or tail. On the other hand, when the twin bodies come together by ventral rather than by bilateral union, the formation posteriorly of a perfect single body or tail becomes impossible, since the necessary readjustments of right and left structures in the twin embryos can no longer take place.

We see then that Gemmill is quite uncompromising in his position that double monsters of all sorts are fusion products. We have already pointed out that the fusion theory is quite untenable and shall attempt to show later

that the dichotomy or fission theory much more nearly satisfies the conditions observed.

Undoubtedly a certain amount of secondary fusion of external adjacent structures does occur, as for example in such cases as that described by Windle in which the tails, crossed the one over the other, are united for a short distance by the fusion of the ventral side of one with the dorsal side of the other. Apart from such obvious cases and such cases as those referred to on page 48, where adjacent identical twins come to fuse side to side by the body wall, we may safely abandon the fusion theory of the origin of true double monsters.

STOCKARD'S THEORY OF THE MODE OF ORIGIN OF TWINS AND DOUBLE MONSTERS IN FISHES

In his recent monograph Stockard (1921) undertakes to explain the morphology and physiology of twinning in the fishes, the basis of his theory being certain experiments in artificial twin production in Fundulus. The starting-point of his work was evidently my own theory of the causes of twinning in the armadillo. He also adopts Gemmill's theory of the origin of double monsters, Patterson's "budding theory" of twin origin, and Child's theory of dominance and subordination.

In so far as Stockard's theory depends upon Gemmill's idea of origin of double monsters by fusion and upon Patterson's budding theory of the origin of armadillo quadruplets, his arguments and conclusions are unsound. Quite apart from the fact that his theory has been erected upon these two insecure foundations, Stockard's own extensions and applications of these theories require additional scrutiny.

Starting with Gemmill's idea that in fishes the germ ring may be regarded "as a stock, able to give rise vegetatively, so to speak, to more than one embryo," Stockard builds up a theory of twinning which I may call the "accessory budding theory." The analogies are drawn largely from the plant world. In Bryophyllum, for example, it is known that the notches around the border of the leaf have the power to bud and give rise to new plants. As a rule, under ordinary atmospheric conditions, only one or two notches give off new shoots. The presence of these shoots seems to inhibit the appearance of others. If, however, these other notches are isolated, each may produce a new shoot.

The periphery of the blastoderm in the eggs of the bird and mammal or the germ ring in a teleost's eggs is probably in some sense comparable to the notched border of the budding leaf. . . . . There are many potential points around the germ ring at which an embryonic axis might arise. Here again, as in the plant, when one bud or embryonic axis has arisen, it tends to suppress the potential ability of other points to form an axis, and normally only one individual is developed in the egg.

We are entirely unable to state the reasons why a certain point along the germ ring should form the bud and not another. One can only imagine that this point has some peculiar advantage of position which gives to it a higher power of oxidation and a temporarily more rapid rate of cell proliferation than is possessed by other points, just as the notch which is dipped below the water surface possesses a budding advantage over the other notches around the leaf. Can the advantage of position possessed by a particular point of the germ ring be reduced so as to equalize the budding tendency of several points and thus allow them to express their ability to form embryonic axes? Could such a condition be brought about, double embryos, twins, triplets, etc., would be produced.

The chief criticisms of this point of view are these:

a) The germ ring is not a stock from which buds appear, for, long before there is any germ ring the axis of the single or of twin embryos is already established. The situation in the bony fish is similar to that in the starfish. The embryonic axis is established at an early blastoderm stage, as has been shown by the analyses of Dr. Hyman (1921). One can readily determine that one side of the blastoderm is the posterior end, or the end at which gastrulation will occur. There is no such thing as a germ ring until long after the blastoderm exhibits definite axiate relations. When two embryonic shields arise they have originated through a breaking down of the original polarity and a physiological isolation of two regions of gastrulation on the blastoderm before any germ ring is present. When the germ ring appears it is merely a non-embryonic region of the blastoderm, concerned primarily with yolk overgrowth, and it ultimately forms merely the neck of the yolk stalk.

b) If the early blastoderm, long before germ-ring formation, already has a definite anterio-posterior axis and the point of gastrulation is well established, we can hardly agree with Stockard's assertion that "we are entirely unable to state the reasons why a certain point along the germ ring should form the bud and not another." Surely the location of the region of gastrulation is not due merely to "some peculiar advantage of position which gives it a higher power of oxidation and a temporarily more rapid rate of cell proliferation." Gastrulation is the same process wherever it occurs, and it takes place at points predetermined by the original polarity of the egg. One only need refer to the case

of the starfish again to make this clear. Under normal conditions the point of gastrulation is exactly at the vegetal pole or the posterior end of the primary axis and this point is fixed, in all probability, before cleavage begins.

c) If one wishes to stretch the concept of budding so as to make it include the process of gastrulation it might be legitimate to refer to additional archentera or points of gastrulation as accessory buds. Personally, however, I see no reason for viewing as a process of budding the ingrowth of the archenteron. One might equally readily consider any other point of rapid growth as a bud. If we are unwilling to accept the term budding for the normal process of gastrulation, it seems hardly feasible to use this term for additional centers of gastrulation.

d) The question now arises as to whether all fish twins, as Gemmill and Stockard believe, result from separate points of gastrulation (embryonic axes). It seems to me highly probable that many, possibly all, twins which are separate at the two extremities and united only in the middle region by external connections, do actually arise from the physiological isolation of separate embryonic shields. If the original polarity of the blastoderm be largely obliterated two secondary growing-points of equivalent value, or possibly of slightly different values, may arise, neither one of which is dominant over the other. From such twin axes will develop the types of twin fishes which we have previously classed as separate twins. If, however, the original polarity is only slightly weakened so that a secondary area of gastrulation arises at the opposite side of the blastoderm, such an area is likely to form a small embryonic shield

which gives rise to a smaller, somewhat inhibited embryo that may subsequently come to be hardly more than a parasite on the body of the primary individual. This would be my interpretation of most of the cases of parasite and autosite described by Stockard and others (Figs. 25 and 26). Windle has cited a case in which the parasite has been reduced to a mere bump on the side of the autosite. If we were using the terminology of budding it would be fair to consider such a parasite as the product of a "secondary bud," because the primary "bud" has retained its identity. When, however, the twin axes are both new and equivalent, neither would be a secondary "bud." Though, as we have seen, the budding conception seems far fetched and valueless, it is relatively unobjectionable when restricted to separate twins. It is when this concept is carried over to the field of conjoined twins that we find it utterly untenable.

THE LATERAL BUDDING THEORY OF THE ORIGIN OF CONJOINED TWINS

Stockard notices, as had many others before him, that the two components of a pair of conjoined twins are frequently of unequal size. As a rule the larger component is practically normal, while the smaller exhibits various evidences of having suffered inhibition. Such smaller components frequently show the same types of abnormality (cyclopia and similar defects) as are seen in single embryos that have been exposed at an early period to growth-retarding agents. Stockard interprets this situation somewhat as follows. The normal component is thought of as arising from a primary embryonic shield which would have formed a single embryo but

for the interruption of development. The abnormal component is viewed as the product of a secondary or lateral bud. An analogy is presented between this condition and that seen in certain plants with a terminal growing-point. So long as the terminal growing-point (equivalent to the primary bud or the normal embryo) retains its normal rate of growth, secondary buds are inhibited; but if the primary bud be injured or removed, secondary buds (equivalent to the smaller embryo) arise and grow, but are often partially inhibited by the presence of the primary bud. This theory seems vaguely to imply that the smaller component arises in some way from the side of the primary axis like a lateral branch, while the original head remains intact as the head of the larger component. Possibly, however, no such crude analogy is intended, but we are merely meant to infer that a smaller "secondary bud" or embryonic shield arises on the germ ring and that through the process of concrescence the primary and secondary individuals fuse in such a fashion as to give us individuals duplex anteriorly and simplex posteriorly. Whichever of these alternatives is meant, one is as untenable as the other. The lateral budding idea is quite incompatible with the fact that even when two components are distinctly unequal they contribute quite symmetrically of all their median organs to the single part of the body. Such an idea would involve the assumption that budding began internally so as to involve notochord, neural tube, and all other median structures, and that these structures divided equally between the stock and the bud—a process that would be not budding at all but fission. The second alternative involves the old-fashioned view

of embryo-formation by concrescence and a secondary fusion of originally separate embryonic axes, a view which we have already shown to be discredited.

How then can we explain the apparent partial suppression of one component? Two simple explanations come to mind. The first is suggested by a study of the interinfluences of human one-egg twins (see chapter x). The commonest mode of interinfluence which may be detrimental to one or both twins is shown to have to do with anastomoses of the placental blood vessels. Now Coste, as long ago as 1855, showed that frequent anastomoses occur among the vitelline blood vessels of fish twins and double monsters. Sometimes pronounced inequalities were noted in the relative sizes of the vitelline veins of the twins. The hearts of twins frequently beat alternately so that there might occur back pressures through the anastomoses. These observations on fish twins seem to imply that the opportunities for one twin to injure the other through the circulation are as good as they are in the case of human twins; and how much injury may be done in the case of the latter is hereinafter abundantly shown.

The second explanation really implies the adoption of the fission theory of the origin of double monsters. This theory maintains that such conjoined twins always arise through the separation, more or less extensive, of the two bilateral halves of a single embryonic shield. It is held that the separation of the two halves of the axis is due to a lowered rate of metabolism at the time when the axis is being established, i.e., during the time just preceding gastrulation. The primordium of one half of the axis may, after physiological isolation from

the other, be more severely inhibited and hence develop less normally than the other. The question arises as to whether we have any evidence that such a physiological asymmetry of the bilaterally equivalent primordia exists. There is in abundance exactly such evidence. Anyone who has engaged in the experimental production of monsters in fishes cannot help but recall that one of the commonest types of deformity is unilateral in its occurrence. One finds many embryos with a normal and a subnormal eye, with one pectoral fin smaller than the other. Another very common type of deformity is that in which one whole side has been relatively suppressed so as to cause the animal to have a curved or spiral axis. Now, if in a single untwinned individual one side may be inhibited while the other remains normal, there is no difficulty about explaining the difference between the bilaterally placed components of double monsters. When once one component becomes relatively inhibited it might be secondarily further suppressed by the stronger twin through the medium of the circulation and might ultimately be almost or quite obliterated. Several instances have been cited, both in human twins and in fish twins, in which such a relatively inhibited component of a true double monster is seen to be reduced to the condition approximating that of a parasite on the body of the normal component.

THE FISSION THEORY OF THE ORIGIN OF CONJOINED TWINS IN FISHES

This theory depends on the conception that a bilateral organism is in a sense a dual individual. A limited amount of concrescence obviously occurs even in forms

in which the germ ring plays no part in the formation of the embryonic axis. This means that the two bilateral primordia are formerly more or less separate and that at the time of gastrulation, or the formation of the axis of symmetry, there is a highly energetic coming together of the cells of the two sides so as to converge in a median dorsal line. The energy of concrescence is greatest at the anterior end, as may be determined by susceptibility experiments, and progressively less great farther down the axis. If at the time when the process of concrescence is going on most actively the rate of metabolism is markedly lowered, or even if the actual interruption of development has been earlier and its effect still persists at the time of gastrulation, this process may be more or less inhibited. If the inhibition is slight it may affect only the most susceptible structures such as the forebrain and the eyes; if a little more severe, certain dorsal or median anterior structures may be prevented from concrescing; if considerably more severe, the effect may be felt far down the axis and all structures except certain median ventral ones may be divided. This view is entirely consistent with the facts. It rationalizes the symmetrical arrangements of the divided primordia, for if the notochords or neural tubes are the products of the bilateral fission of a single primordium, what more natural than that they should be equal and symmetrical? It rationalizes the observations that *situs inversus viscerum* is of very common occurrence, for if the two components of conjoined twins are derived from the bilateral primordia of a single individual we might expect to find such evidences of mirror-image symmetry. This view is essentially one of the physiological isolation

of bilateral primordia through suppression of the active focusing of equivalent bilateral anlage upon a single median line.

Cases of more or less complete isolation may occur. The minimal cases are probably those involving minor degrees of discoördination and consequent asymmetry of the two sides, as in fish embryos in which one side grows and develops less well than the other. It seems likely that the condition known as hemihypertrophy in man (chapter xi) is to be viewed also as a case of minimal twinning. The maximal cases are those in which the two components are entirely separate except for a common anus or a common median ureter. It may also be true that some completely separate fish twins arise by simply going a short step farther than the last-named condition. Unless this is true it would be difficult to account for the occasional cases of symmetry reversal in one of a pair of completely separate twins. It is my opinion, however, that the great majority of separate twins come from separate embryonic axes and that all true conjoined twins arise by the dichotomy or fission of a single embryonic axis.

THE EXPERIMENTAL PRODUCTION OF TWINS IN FISHES

Gemmill expresses the opinion "that the occurrence of double monstrosity (twinning) is due in the main not to environmental factors, but to conditions which are inherent in the germ cell." In another connection he says: "The likelihood cannot be excluded that external factors sometimes induce the production of double monstrosities in the developing eggs of fishes."

Farther than a realization that twins might be due to environmental changes, Gemmill did not go. His attitude is essentially that of a pure morphologist of the old school and not that of an experimenter.

Stockard, however, taking his cue from my own theory of the cause of twinning in the armadillo, undertook with some degree of success to produce fish twins by lowering the rate of development of the early embryo. He found that by putting recently fertilized eggs of Fundulus into a refrigerator for fairly long periods and then bringing them back to normal temperature, he obtained a few double monsters. Since twins in Fundulus are extremely rare there can be no doubt but that twinning was induced by the cold. Stockard got similar results in a few instances with lack of oxygen. The common factor was, as he calls it, "arrested development." He feels that there is a very intimate relation between twinning and the process of gastrulation, but is rather vague about what this relation is. In one place he says: "Either stopping development or greatly reducing its rate during cleavage or before the germ ring has formed, that is, at periods preceding gastrulation, frequently serves to cause doubleness in the subsequent embryo formation." In another place he writes: "The origin of two embryonic axes or growing-points on the germ ring of the fish probably results from a rather mild or slight reduction in the normal developmental rate at the time of gastrulation or embryonic shield formation." We are thus left in doubt as to the critical period for twinning. Stockard seems to waver between two positions and it is uncertain whether he considers the critical period before or during gastrulation. He has,

however, rendered a distinct service to our understanding of the causes of twinning by his experimental demonstration that twins may be produced experimentally by lowering the developmental rate. Just why some eggs twin while others remain normal and still others in the same batch exhibit various types of single deformity we do not at all know. We have known for a long time, however, that eggs are highly variable in their susceptibilities to various inhibiting agents. It probably requires just a certain degree of susceptibility in an egg and a certain degree and duration of inhibition to give the particular result we call twinning; and just the right combination of the two variables does not often occur in Fundulus.

Evidently in trout the frequency of twinning is greater, but even there the percentage of twins is very small, for there is hardly more than one twin in 1,000 eggs under the conditions prevailing in fish hatcheries. Doubtless, however, if Stockard's methods were tried on the trout or the salmon, relatively large numbers of twins would be produced.

CHAPTER VI

TWINNING IN BIRDS

There is a voluminous literature on twinning (duplicity) in birds. The egg of the domestic fowl has for a long time been a classic object for the study of vertebrate embryology and many thousands of embryos have been incubated and examined every year in all parts of the civilized world. Doubtless every embryologist who has handled any considerable number of chick embryos has encountered one or more cases of duplicity. As a result of this, numerous papers have been published about double monsters in the chick, and a great many examples of various kinds of duplicity have been figured.

THE WORK OF DARESTE

The classic treatise on double monsters in birds is Dareste's *Production Artificielle des Monstruosités* (1891). This volume marks a new era in the scientific study of teratology, for the method is thoroughly experimental. By manipulating the temperature, humidity, and oxygen conditions at various stages of embryonic development he produced all of the regulation types of single monstrosity and developed a theory of teratology which has been adopted and extended within recent years by Stockard. He believed that the great majority of monstrosities are developmental arrests in the sense that certain organs at critical periods in their development are permanently halted and fail to reach the normal definitive condition. Each organ or system has some especially susceptible

period when it is readily arrested. A few anomalies are interpreted by Dareste as the result of developmental excess, a sort of supernormal development. Some types of monsters are interpreted as the result of adhesions and of unions of similar parts.

The part of Dareste's treatise which especially interests us at present is that in which he deals with the origin of double monsters and twins. He reviews the history of theories of the origin of double monsters, theories that date back as far as the beginning of the eighteenth century.

The anatomist Duverney in 1706 published an account of the organization of an ischiopagus human monster and expressed the conviction that it could not have arisen through the partial fusion of two embryos but must have pre-existed as a double monster in the egg. He was imbued with the prevalent doctrine of preformation and therefore found the idea of a completely preformed double monster more acceptable than one involving epigenetic changes.

In 1724 Lemery, on the basis of another dissection of a two-headed, single-bodied human double monster, sought to prove that such monstrosities could not have been preformed but must have resulted from two separate embryos derived from two eggs.

Winslow came to the support of Duverney's position and opposed the fusion idea on the grounds that the double monsters were symmetrically united and that one of the components showed *situs inversus viscerum*, a condition impossible to account for on the basis of the fusion theory.

Wolff (1772) combated the doctrine of preformation and revived the epigenesis doctrine of Harvey. He

agreed with Winslow's objections to the idea of fusion and proposed the theory that double monstrosity was determined through some peculiarity of the process of fecundation.

Meckel, who was a follower of Wolff, carried the epigenesis conception still farther and concluded that all double monsters are cases of developmental excess, inasmuch as they are derived from a single egg. All supernumerary parts are conceived of as the result of a complete doubling of a particular organ. This doubling might involve only one finger or the whole body, as in the case of twins. He believed in a dual origin of a bilaterally symmetrical animal, and that in double monsters the two halves fail to unite or unite only partially. Like Wolff he sought to explain the doubling as the result of a peculiarity of the process of fecundation such as double fertilization or some other irregularity.

In 1826 Etienne Geoffroy Saint-Hilaire revived the fusion idea of Lemery, but instead of supposing that the fusion was a purely accidental phenomenon, he tried to explain the striking symmetry of monsters by stating that homologous organs have an affinity for each other and only like parts would fuse with like. Saint-Hilaire did not follow up this hypothesis but passed it on to his son, who developed the idea much farther. The latter urged against the theory of Meckel such cases as double pelvis, double breasts, double faces, which, however, seemed to him easily explicable on the basis of the hypothesis of original duality.

So influential was the fusion theory of the two Saint-Hilaires that it has been adopted by Dareste, by Gemmill, and by Stockard.

Dareste, however, made a distinct advance in that he adopted for study the bird egg where, if anywhere, he should have been able to see double monsters in the making. He found a number of cases of two or more blastoderms on a single egg; not only that, but two embryos upon a single blastoderm. He noticed that these paired embryos on one blastoderm often lie symmetrically with reference to each other and this to him seems to make the idea of double monsters as fusion products entirely reasonable. On these grounds he adopted the theory that double monsters are the product of the fusion of two embryonic axes that have arisen on a single blastoderm. He favors the theory of the Saint-Hilaires that like part tends to fuse with like, though there are absolutely no grounds for such an assumption.

As to the causes of twinning Dareste has no theory. One would think that a man so imbued with the idea that monstrosities are all due to developmental disturbances might readily have concluded that double monsters were so produced, but he did not seem to take this step. On the contrary, he specifically states that twins and double monstrosities are predetermined before laying through some disturbance of the process of fertilization. For him double monsters are not in the same category with the numerous types of single monstrosity which he describes so much in detail.

MODES OF TWINNING IN BIRDS

Since the appearance of Dareste's treatise a large literature concerning double monsters in birds has grown up, and many excellent figures of double monsters of

many kinds have been published. A survey of a large number of these figures and of sections through crucial parts of double embryos, together with a study of a collection of several pairs of chick twins in my own possession, has convinced me that the situation is essentially the same as that described for the star-fish; that the modes of twinning and the different categories of twins are essentially the same in two cases. I find in the birds the following kinds of twins: (1) those derived from two blastoderms on a single yolk; (2) those derived from two embryonic axes on one blastoderm; (3) those derived by the isolation, more or less complete, of the bilateral halves of a single embryonic axis; (4) those derived by the growing together of two separate embryonic axes; and (5) those in which one component of a double monster is more or less completely absorbed by the other. These five categories of twins will be discussed seriatim.

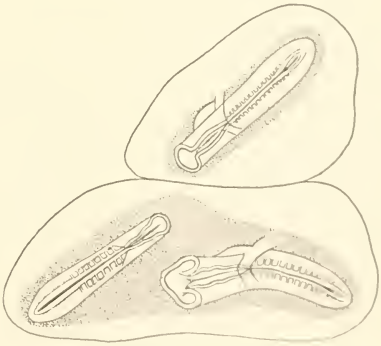


FIG. 34.—A case of triplets in which the upper individual probably arose from an early fission of the blastoderm into two unequal masses. The lower pair of twins is obviously the result of double gastrulation of the larger moiety of the divided blastoderm. (After Dareste.)

1. That separate blastoderms on a single yolk actually do occur seems certain in view of the clear figures of Dareste and of Kaestner. That of Dareste (Fig. 34) represents a

case of triplets, two embryos derived from one blastoderm and one from the other. The twins, seen in the lower part of the figure are believed to have been derived from one blastoderm as the result of double gastrulation. Though these two embryos are in all probability the result of two secondary areas of gastrulation and are therefore bilateral equivalents, one is considerably more advanced than the other. Such examples of unilateral asymmetry are no more difficult of explanation than are cases in which one side of a single embryo develops more rapidly than the other. A clearer case of twin chicks derived from two separate blastoderms is one presented by Kaestner (Fig. 35). This case consists of a pair of twins in the primitive streak stage. Here we have a good example of a blastoderm which has undergone bilateral fission so as to form two rather unequal blastoderms. The fact that the two embryos, though of very unequal size, bear a mirror-image relation to each other, is significant.

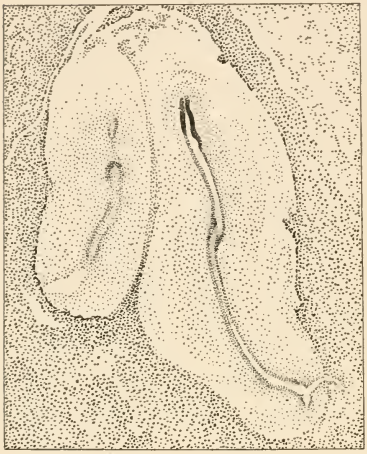


FIG. 35.—An early stage of twinning in the chick, evidently the product of an unequal fission of an early blastoderm. (After Kaestner.)

2. Separate twins arising from two points of gastrulation on a single blastoderm are usually symmetrically placed so that the anterior ends of the axes tend to

approach each other toward the center of the blastoderm. Sometimes the two embryos remain entirely separate, at least for a time. Kaestner (1901) figures a twin chick in the primitive streak stage in which the two axes were in the same line, much like the twin starfish shown in Figure 6, but the anterior ends are still some distance apart. The same author presents a very clear photograph (Fig. 36) of a similar pair of twin embryos in a thirteen- or fourteen-somite stage, with

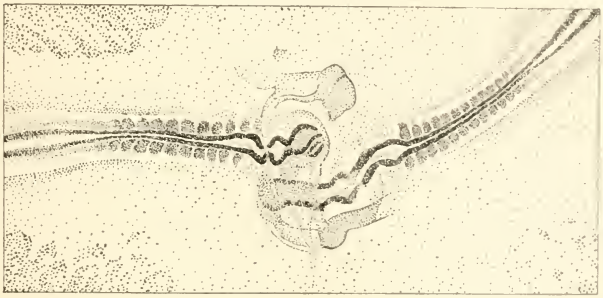


FIG. 36.—A rare type of chick twins that are the product of double, symmetrical gastrulation. The two embryonic axes have grown inward toward each other and have barely avoided a collision such as has taken place in the twins shown in Fig. 39. (After Kaestner.)

axes almost in the same line. Their heads have, however, shoved past each other as far as the hind-brains. Though there is some contact and confusion in the membranes, especially the amnia, of these twins, they are entirely separate and have, in my opinion, arisen as two quite distinct twin areas of gastrulation on opposite sides of the blastoderm. This opinion is strengthened by a study of a very interesting double monster in my possession (Fig. 37). In this case it is obvious, I believe,

that the two inwardly growing embryos have met each other head-on instead of growing past each other as in Kaestner's case. On this account there is a considerable amount of crumpling up of the brains and other anterior parts. The heart of one has been crowded off to the left and that of the other, to the right. Such a duplicity as this cannot have arisen as a fission product of a single

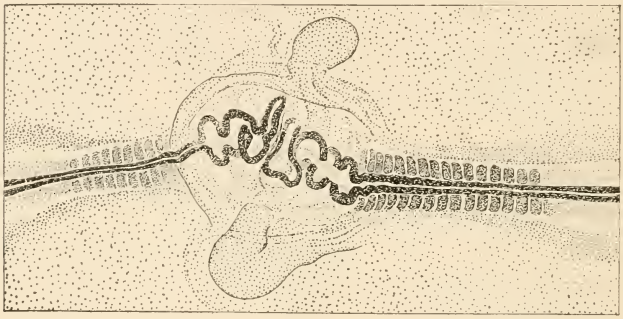


Fig. 37.—The result of a head-on collision between chick twins that have resulted from double, symmetrical gastrulation on opposite sides of the blastoderm. The results of the collision are seen in the crumpled forebrain and displaced hearts. This case is equivalent in mode of origin to the type of *Patiria* twin shown in Fig. 7. (Original.)

embryonic axis. Dareste, Kaestner, and Tannreuther have described cases similar to this, but such purely mechanical fusions as this are quite different in character from most double monsters, and must not be considered as supporting the theory that cosmobia (truly symmetrical double monsters) are the product of the symmetrical fusion of paired embryonic axes.

A really crucial case has recently come into my hands through the kindness of Dr. B. H. Willier. This is a

double-monster chick (Fig. 38) in which there is apparently a fusion in the head region. The two bodies are very symmetrically placed and practically in the same developmental stage (about seven somites). As compared with normal single embryos of this age we note

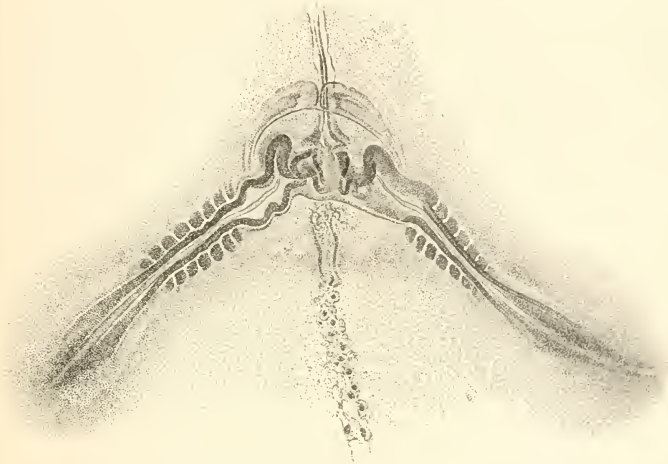


FIG. 38.—A practically symmetrical pair of secondarily fused chick twins, resulting from a collision at a tangent instead of head-on as in Fig. 37. It is such cases as this that have convinced some writers that true katadidymi are the result of the symmetrical fusion of separate embryonic axes. (Original.)

decided differences. Though the brains of the two components are fairly symmetrical, there is obvious crumpling of both. No part of a normal single head could possibly arise from such a condition. No omphalomesenteric veins are present on either component, while the anterior vitelline vein of one embryo has developed to one side and that of the other to the other side. This condition also could never so regulate itself as to give

a normal single condition. We may conclude then that *true double monsters (cosmobia) cannot be derived through the coming together at their anterior ends of separate embryonic axes.*

In this category of twins we may include the very rare case of Dareste (Fig. 39) consisting of a set of triplets upon a single blastoderm. The facts that the heads all point inward toward the center and that each lies on its left side like a normal single embryo, argues for their derivation from three separate and equivalent points of gastrulation. No other similar condition has ever been described.

FIG. 39.—A fine example of identical triplet chick embryos derived from triple gastrulation of a single blastoderm. (After Dareste.)

3. A third type of twin chick embryo is that which has obviously arisen through the separation of the right and left primordia of a single embryonic axis. Embryos of this sort are not at all uncommon. One case of Tannreuther's that seems to me to bear this interpretation is that of a three-somite stage in which there are two entirely separate heads and the body, back to the posterior end of what was originally the head process, is symmetrically divided into

two equivalent primordia (Fig. 40). The three mesoblastic somites in the middle seem to belong in common to the two embryos. The whole primitive streak region seems to be single. In brief, the head-process region back to the primitive knot has twinned and the post-cephalic region has remained untwinned. Such duplicities are technically called anadidymi.

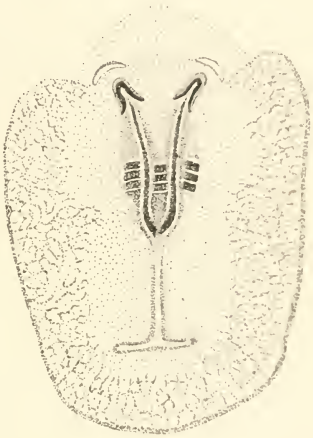


FIG. 40.—A typical double-headed chick monster (anadidymus) resulting from the partial dichotomy of the anterior end of the originally single embryonic axis. (After Tannreuther.)

As is the case in human double monsters there is a class of chick duplicities in which the head region is more or less completely single while there are two widely diverging trunks. A typical example of this sort of monster, really a katadidymus, is figured by Tannreuther (Fig. 41, p. 84). The head region back to the somites is normal and single; posterior to that there are two bodies diverging at an angle of about 120°. Tannreuther considers that this "double embryo no doubt began its development as two independent primitive streaks, with a later connection or fusion of the anterior ends of the two-head processes." With this conclusion I cannot agree. It seems to me inconceivable that two separate axes could come together with such perfect symmetry as to form a normal single head. In brief, the objections to the

fusion idea are the same in this case as in other cases previously considered. The condition is, in my opinion, unquestionably a result of a process of fission of the posterior growing region of the embryo. It must be

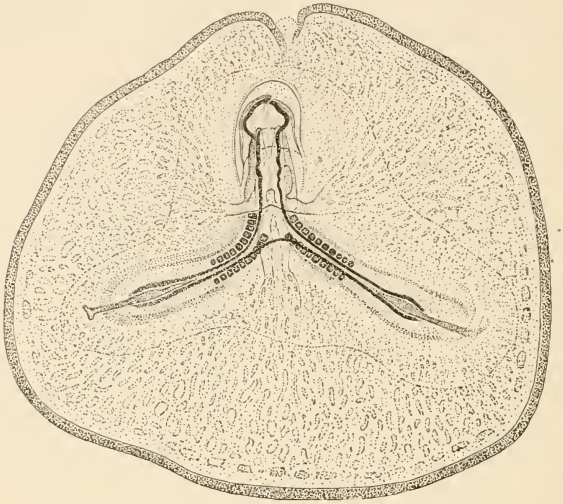


FIG. 41.—A typical two-tailed chick double monster (katadidymus) due to the partial fission of the posterior end of a single embryonic axis. (After Tannreuther.)

supposed that at the time when the agencies responsible for twinning were applied, the head parts of the symmetry axis had been definitely established and that there was a secondary point of great activity and high susceptibility at the level of the dorsal lip of the blastopore which is probably at the anterior end of the primitive streak.

In the chick there appear to be really two distinct regions of differentiation and two gradients: one the head process and the other the primitive streak. The

latter is believed to be the equivalent of the closed blastopore and its anterior end is the dorsal lip region, equivalent to that described as a secondary point of high susceptibility in the frog. It appears that either one of these regions may undergo fission without the other or that both may undergo fission more or less completely.

Kaestner (1898) describes and figures an interesting case of a double chick embryo in approximately the same stage as that of Tannreuther shown in Figure 41, but the head, instead of being a normal single region, is much broader than usual and is partially double, the forebrain being single and the rest of the central nervous system being double. Such a condition is interpreted as a case of partial fission of the head-process region of the blastoderm. Whether it is possible for two entirely separate chick embryos to arise through the fission of the bilateral primordia of a single embryonic axis is a question at present unsettled. Tannreuther shows an example of twin chicks that I am compelled to interpret as products of a nearly complete fission. As may readily be seen (Fig. 42, p. 86) the two embryos are entirely separate except at the very posterior end where, back of the tail-bud region, the two primitive streaks unite for a short distance. Unless some sort of gross concrescence of the posterior margin of the blastoderm has occurred, this single region of the primitive streak can be explained only as the residue of an originally single embryonic axis that has undergone almost complete bilateral fission. In this blastoderm the two embryos are markedly different in size, the right-hand one being much larger and more advanced (fourteen somites), while the smaller one is in a ten-somite stage. Tannreuther is of the opinion, and

in this I agree, that in this case "two distinctly independent chick embryos would have resulted at the end of the incubation period."

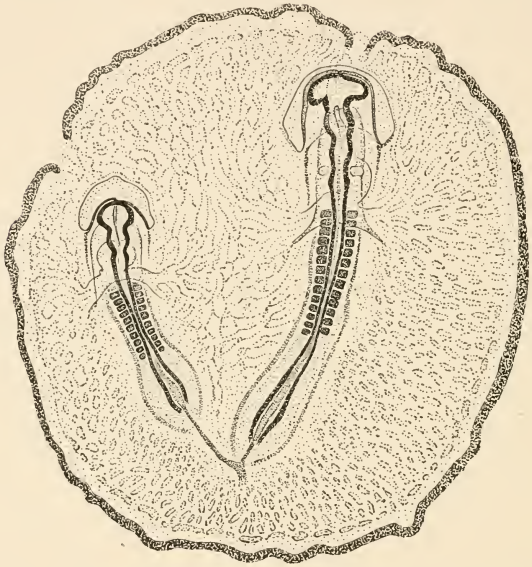


FIG. 42.—A rare type of chick duplicity, probably the result of a nearly complete longitudinal fission of an originally single embryonic axis. There are no evidences of plural gastrulation but, on the contrary, the posterior end of the axis is still single. (After Tannreuther.)

4. Already under the second heading I have indicated that there is some very good evidence that twin embryos originating from separate embryonic axes and arising directly opposite to each other on the same blastoderm, may grow toward each other, meet head on, and fuse at their anterior ends (Figs. 37 and 38). I see no other satisfactory explanation of the crumpled condition of the two anterior ends. Examples of such a condition have already

been described on pages 79–81. In these embryos the brains are much folded and wrinkled as though each had interfered with the other's growth.

5. Many authors have described advanced twin embryos in which the autosite and parasite condition is obvious. In my opinion this condition may arise from either of the two categories numbered (2) and (3), but is much more likely to arise from (2). An unusually interesting case (Fig. 43) of autosite and parasite in the making is one in my own possession. This curious pair

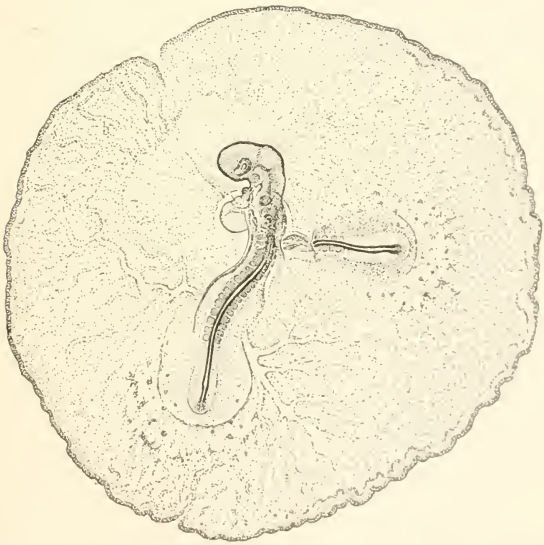


FIG. 43.—A very unusual type of chick twin embryo, doubtless a case that would lead to the autosite-parasite condition. The two embryos probably arose from two separate points of gastrulation, one of which was primary and the other secondary. The smaller, secondary, axis has evidently been partially inhibited by the larger, primary, axis, and was destined to be a mere parasite on the body of the latter. (Original.)

of chick twins are of very unequal size. The large embryo is quite normal and has seventeen somites. The small embryo, which is quite separate as yet from the large one, is in about a four-somite stage and is therefore some eighteen hours less advanced. The head end of the small embryo nearly touches the body of the large one and its axis is at right angles to the latter. The vitelline circulation of the large embryo is seen to be invading the vitelline area of the small one and it is highly probable that the small embryo would later have become a mere cyst on the side of the larger or might have been totally absorbed. If one were to attempt to assign this double embryo to one of the above-mentioned categories he would be compelled to select number 2, for there is no evidence that the two are derived from separate blastoderms. These twins are, therefore, the product of unequal double gastrulation.

THE CAUSES OF TWINNING IN BIRDS

Stockard points out that:

> The eggs of birds normally have a discontinuous mode of development. Fertilization takes place in the upper part of the oviduct and the egg begins its development in the high temperature of the maternal body and continues to develop as it travels down the uterine tube and becomes surrounded by its accessory coats. Finally at the time of laying, the blastoderm has passed the gastrula stage. The fall in temperature experienced on leaving the body of the mother causes development to stop in this early post-gastrula condition, and the egg remains quiescent until the temperature is again raised to about that of the bird's body.

It is believed that the reason why the sudden and rather prolonged interruption of development so seldom results in twinning is that the critical period for twin-

ning, the period of gastrulation, has passed in most of the eggs before they are laid. Only a few eggs that are laid prematurely, before the process of gastrulation has been completed, undergo twinning. Patterson (1909) has shown that there is considerable variability in the state of advancement of eggs at the time of laying. Certain hens have a greater tendency than others to deposit eggs before gastrulation is complete. If this trait is hereditary it would not make much progress in the race because the prematurely laid eggs are very likely to develop non-viable double monsters or other abnormalities. By a process of natural selection it has probably resulted that only strains of birds with an inherited tendency to lay the eggs relatively late have survived.

Although we have no definite data on the artificial production of twins in birds the prevalence of twins reared under artificial conditions implies that the twinning process is the result of some interruption of the normal course of embryonic development that results in the partial deaxiation of the blastoderm and thus permits of double gastrulation or else causes the physiological isolation of the bilateral primordia of a single embryonic axis. The fact that the vast majority of bird twins are of the conjoined rather than of the separate type leads to the conviction that the former are as a rule the product of a relatively late developmental interruption involving the final steps in the establishment of the embryonic axis. An earlier retardation would be expected to cause the fission of the head process and a later retardation that of the primitive streak or posterior parts. This would seem to be a rational explanation of

the relatively marked prevalence of katadidymi among the birds, for this is the commonest expression of twinning in the group, in striking contrast with the situation in the fishes where anadidymi are the almost universal type and katadidymi extremely rare.

All of these facts accord with the phenomenon previously referred to: that in the birds developmental interruption at the time of laying occurs only after the process of gastrulation has made considerable progress. We would expect, then, and actually do find that twins from separate blastoderms are very infrequent, that those derived from two gastrulations are next in rarity, that anadidymi come next, and that katadidymi are the most frequent.

CHAPTER VII

TWINNING IN AMPHIBIA, REPTILES, AND OTHER CHORDATES

AMPHIBIA

Twinning appears to occur with extreme rarity among the Amphibia. The fact that normal development takes place at very low temperatures may partially explain the failure to twin; for low temperature seems to be the main reason for twinning among egg-laying vertebrates other than the fishes. Twinning may readily be induced among the Amphibia, however, by experimental procedures.

O. Schultze (1894) showed in the case of the frog that if the egg is inverted while it is in the two-cell stage of cleavage in such a way that the white or vegetative pole is turned upward, each blastomere will give rise to a whole embryo. Figure 44, page 92, shows several of his double embryos at various stages of development. Morgan (1901) in commenting upon these results says: "In this case it appears that the results are due to a rotation of the contents of each blastomere so that like parts of the two blastomeres become separated." There is also doubtless another reason for the physiological isolation of the two hemispheres, viz., retardation, due to the long delay consequent upon the necessity for each blastomere to undergo a complete reorganization of its axiate structure. Each cell, after the temporary cessation of developmental activity, starts out for itself as though it were a separate egg, and each produces a whole embryo.

Tornier has performed a number of experiments on the salamander, Triton, involving artificial doubling of tails and limbs, using the methods of constriction and cutting off of parts. This mechanical isolation of growing regions results in the same types of doubling of limbs and tails as does physiological isolation in other forms.

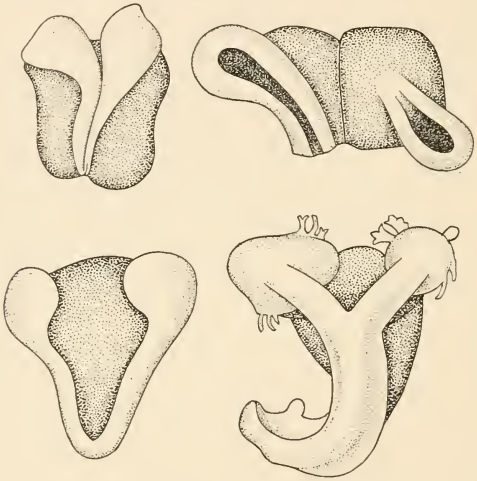


FIG. 44.—Types of double frog embryos, due to inverting the eggs in the two-cell stage. (After Schultz.)

Spemann and Falkenberg (1920) have recently presented some very interesting data concerning the artificial production of twins in Triton. Early gastrula stages were cut in two along the sagittal plane so as to make two equivalent right- and left-hand half-embryos. These as a rule regenerated the lost half, giving rise to artificial monozygotic twins. About half of the right-hand pieces showed *situs inversus viscerum*, or reversed symmetry of the heart and stomach. This interesting

case of physical isolation of the equivalent halves of a single embryonic axis closely parallels what we believe occurs through physiological isolation in other groups. Inasmuch as this work is to be discussed in a subsequent chapter dealing with the matter of reversed symmetry, we shall omit further details in this place.

Bellamy (1918), in his elaborate series of experiments dealing with the modification and control of development in the frog, obtained one double-headed individual and not a few with single head and more or less completely double bodies and tails. Some of the individuals were double as far forward as the forebrain; others were double only in the tail region. This writer was, at the time of his experiments, not much interested in twinning and did not discuss the matter at any length in his paper. He merely says that "*spina bifida* of all degrees is of course common under conditions that inhibit development, and result primarily from inhibition of the dorsal lip region" of the blastopore. Bellamy thus takes a position, in harmony with my own, that these duplicities which he has merely referred to as "*spina bifida*" are cases of bilateral twinning and are due to the inhibition of a particularly susceptible region of the embryonic axis, the dorsal lip of the blastopore. If this region of growth is inhibited and lateral regions continue to grow, twinning is inevitable. It may be said that the dorsal lip region is probably a near equivalent of the region of the fish embryonic shield called by Kopsch the *Knopf*, which is the primordium of the tail-bud. We have already shown that the tail-bud region is a secondarily acquired point of high metabolic rate and very active growth. Such a region might readily be so inhibited as

to bring about the physiological isolation of two points somewhat separated from the median point—that point most strongly inhibited—and the consequent production of two potentially equivalent tail-buds.

An interesting case of minimal twinning, similar to that described for sea-urchin and starfish larvae in our chapter on symmetry reversal, is described by Bateson (1894) for a tadpole of *Pelobates fuscus*. Instead of having one spiracle on the left side, as is normal for Amphibia, there are paired spiracles of equal size. This is cited by Bateson as a case of homoeosis.

REPTILIA

Partial twinning involving a more or less complete doubling of anterior structures is probably fairly common among reptiles; but relatively few workers have given attention to the subject of reptilian embryology and therefore few cases of twins or double monsters have been reported. Reptilian development is so similar to that of birds that this field of embryology has been relatively neglected. That twinning does occur probably with even greater frequency and with more success in the reptiles than in the birds, I have many reasons for believing. While, as Bateson points out, there are no authentic records of a double monster in mammals or in birds having *grown up* in a wild state, there are many such cases among the reptiles. Several of the older and some of the newer, more critical writers have described instances of complete or partial duplicity among the snakes, involving mostly double or triple heads. At least two authentic cases are on record. In some instances the doubling involves only the most anterior

median organs, such as the nostrils. Instances are known of the occurrence of four nostrils, representing an even slighter degree of twinning than the presence of a third or median eye in fishes.

Gemmill (1912) cites a case of a three-headed snake seen near Lake Ontario by Bruch. He also notes that another three-headed snake was reported by Androvandus from the Pyrenees Mountains.

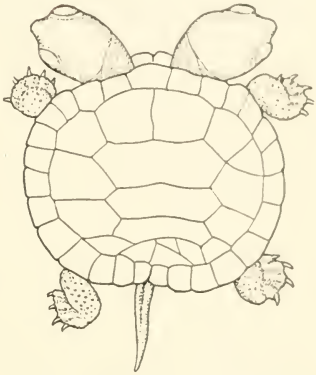


FIG. 45.—Double monster turtle of the anadidymus type. (After Bateson.)

Among Chelonia there are very few recorded instances of twinning. Bateson describes and figures an interesting specimen of two-headed tortoise (Fig. 45) in which the heads behaved independently as though they had distinct individualities. Such a creature might have some difficulty in deciding on a direction of locomotion. This is, so far as I know, the only recorded case of twinning among the turtles, but I am convinced that embryonic twinning is not infrequent. While living for some years on Lake Maxinkuckee in Indiana, a lake plentifully stocked with several species of turtles, I took occasion to study this group in a rather intensive fashion. Among other things studied were the breeding and nesting habits and the general features of the embryology. Many a morning expedition was made to watch the turtles building their nests in the sand and in soil of various characters. Most of the species dig out with their hind feet a vertical

tunnel several inches deep, enlarged into a flask-like chamber at the bottom. The incubation of the eggs depends entirely upon the heat of the sun that may penetrate the soil. The breeding season comes either in May or early June and it is sometimes decidedly cold and sunless during a considerable part of the period of incubation. There can be no doubt that climatic irregularities have a very marked effect upon the development of chelonian eggs in north temperate latitudes. I have examined a great many nests and have found whole batches of eggs dead and decaying, probably killed by a cold spell during the early periods of incubation. In other batches of eggs I have found a very large percentage of embryos abnormal in various respects: some with imperfect eyes; some with heads small and irregular; some with one or more feet lacking or tail lacking; some with deformed carapace; many with irregularities of the scute pattern of both carapace and plastron, and associated abnormalities. All of these irregularities are obviously due to unfortunate developmental conditions—probably low temperatures. In several hundred embryos of various species of turtles examined I have never found a case of twins or even of unmistakable double monstrosity. One type of abnormality, however, that was fairly common was a condition of more or less extensive doubling of the median series of scutes on the carapace. This type of irregularity was found to be closely correlated with a similar doubling of neural plates, which are the broadened dorsal spines of the vertebrae. Not infrequently there was a dichotomous fission of a rib in association with such doubling, and when there are twinned ribs there are usually twinned

costal plates and accessory costal scutes. In view of what we now know about twinning in vertebrates, I am convinced that this strong tendency to form a double median series of scutes and plates in these subnormal turtle embryos is a case of incipient twinning, due to partial isolation of the median dorsal elements of the right- and left-hand primordia of the axis. That the twinning process sometimes goes much farther than this is evidenced by the fact that two-headed conjoined twins, such as that shown in Figure 45, actually occur.

Why, we may ask, does not twinning occur more frequently when the environmental conditions appear to be such as to favor it? It seems to me highly probable that the same reason applies here as in the birds: that the eggs have passed the critical period before they are laid. There is available some direct evidence that the chelonian embryo is a little farther along than is that of the bird at the time of laying. Consequently we might expect only minimal twinning to occur, viz., that in which the already established axis of symmetry is affected so as to bring about a more or less extensive fission of the bilateral primordia, especially those in the median dorsal position. If then scute and plate irregularities are to be interpreted as the result of a minimal phase of twinning, my earlier interpretation of these "abnormalities" as reversions to an ancestral condition (Newman, 1906) will have to be modified, and I shall have to confess that at one time I was less cautious than I now would be as to the interpretation of anomalous or abnormal biological materials as evidence of phylogenetic or ancestral conditions. Most of us have at some time or other fallen into this familiar type of error.

TWINNING IN OTHER CHORDATES

Cyclostomata.—Only one other class of vertebrates remains in which twinning has not already been dealt with: the round-mouth eels. That twinning occurs here as elsewhere among the vertebrates is evidenced by the fact that several papers have been written on twinning in these forms.

Barfurth (1899) has described a case of a larva of *Petromyzon planeri* with two tails. Bataillon has written a note on spontaneous blastotomy and conjoined-twin larvae in the lamprey.

Elasmobranchii.—Dohrn (1902) has described an interesting double Torpedo embryo which is of especial interest because it is so clearly a product of partial fission. There are two complete notochords but only one median row of mesoblastic somites, belonging equally to the two half-embryos. The outer sides of the two half-embryos are quite complete and exactly equivalent mirror-images of each other. Kaestner (1898) reports the finding of two eggs of the selachian Pristiurus which had two blastoderms on a single yolk. In one of the eggs the two blastoderms were of equal size and about one-fourth of an inch apart; in the other egg the two blastomeres were of very different size and in contact as though one had been split off from the other.

Amphioxus.—While Amphioxus is not a true vertebrate it is believed to be the most closely allied of the chordates to the vertebrates. It is therefore of interest to record briefly in this place the well-known work of E. B. Wilson on artificial production of twins in Amphioxus. By shaking the eggs while in the two-cell stage the blastomeres are either entirely separated so as to

form completely independent dwarf larvae, or else they are only slightly separated so that they cease to act in unison and become physiologically isolated so as to form double gastrulae and double-monster larvae. The conditions are quite like those described for the starfish, Patiria.

CHAPTER VIII

THE CAUSES OF TWINNING IN THE ARMADILLOS

In 1909 we (Newman and Patterson) first discovered and studied "specific polyembryony" in the nine-banded armadillo of Texas (*Dasypus novemcinctus texanus*). It was found that this species habitually gives birth to a litter of four offspring, that all members of any given litter are of the same sex, and that the members of any one litter are usually strikingly alike. In 1910 we published a more detailed study of the development of this species in which all stages from the primitive-streak stage to birth were studied. In 1911 we published a statistical study of variation and heredity in armadillo quadruplets and showed that *the members of a litter are as closely similar to one another as are the right and left sides of single individuals;* they have a coefficient of correlation of .9+ as compared with that of ordinary siblings, which is about .5. It was also clearly shown that the quadruplets were arranged in two pairs and that the two individuals of a pair were more nearly identical than are individuals belonging to opposite pairs. In 1912 I studied the oögenesis and ovulation of the armadillo and showed that only one egg is given off at a breeding, for only one corpus luteum was formed in the ovary. The egg was an entirely typical mammalian egg. In 1913 I formulated the first theory ever published as to the causes

of this phenomenon. In a general paper on the natural history of the nine-banded armadillo the view was expressed that the process of twinning was due to a lowering of the rate of metabolism of the early embryonic vesicle, resulting in the "physiological isolation of parts at certain distances from the dominant (apical) region. When such isolation occurs new centers of control arise, which produce buds capable of establishing whole new systems like the original." At that time no facts were available which seemed to account for the lowering of the rate of development of the embryonic vesicle. Certain peculiar bodies, that were identified by a well-known protozoölogist as protozoan parasites, were found abundantly in ovarian oöcytes, and the suggestion was made that these bodies were the probable cause of the developmental slow-down that initiated twinning. Since, however, the protozoölogist in question subsequently withdrew his original diagnosis of the supposed intracellular parasite, this suggested cause of the lowered rate of metabolism had to be abandoned. Up to this time it was known that each set of quadruplets was the product of a single egg, but the exact time and mode of twinning was not definitely known.

We are indebted to Patterson (1913) for giving us a detailed account of the twinning process. He discovered in considerable numbers blastocysts in pre-twinning stages and also found many stages of twinning. First the originally single ectodermic vesicle elongates in the bilateral axis of the uterus and twin thickenings of the apical ectoderm are formed. This is a true twin stage. Then each of the twins divides bilaterally into two embryos, making two pairs of twins, or a set of quadru-

plets. Once these four twin primordia are established, each develops its own amnion, allantois, and placenta; and they remain essentially isolated, though surrounded by a common chorion, till birth.

In his search for still earlier embryonic stages (the late and early cleavage stages) Patterson made a very important observation, the significance of which he failed entirely to appreciate. He began collecting earlier and earlier in the season for several successive years and found no earlier stages, but did find abundant instances of single untwinned vesicles lying free in the uterus. A cytological study of these vesicles showed that they were not developing, since no mitotic figures were to be found in any of the tissues. This "period of quiescence" lasted at least three weeks, and probably longer.

Here then was unequivocal support of my original theory that twinning was due to a developmental slow-down, and I immediately realized the importance of this, but it was not until 1917 that a further elaboration of my theory of twinning was made public. In the volume on *The Biology of Twins* the significance of the "period of quiescence" described by Patterson was discussed, and twinning was explained as the direct result of this period of quiescence. The view then expressed was that, as the result of a very marked retardation in the rate of development, the original apical region lost its dominance over subordinate regions and that, when placentation occurred and development was resumed, at first two new centers of growth or apical points arose, and later two others became isolated; so that, instead of one apical end or head, four head pri-

mordia were physiologically isolated on the originally single ectodermic vesicle. From the time of their isolation till they are born the four individuals remain morphologically and physiologically independent. They are merely inclosed in a common chorion, which antedates the twinning process.

For a considerable time then I have steadfastly held the view that twinning in the armadillo is caused by arrested development resulting in a more or less complete obliteration of the axiate organization, together with loss of the integrative properties of the original head and the emancipation of subordinate regions from the control of the original dominant region. This plainly suggests physiological isolation. I have no reason to abandon this general view, but shall attempt to give to it a more concrete setting.

CAUSES OF THE "PERIOD OF QUIESCENCE"

The progress of a scientific theory is one that proceeds step by step from immediate cause to causes more and more remote. The immediate cause of twinning in the armadillo is the physiological isolation of secondary growing-points (apical points); the cause of physiological isolation is the partial obliteration of the axiate relations in the ectodermic vesicle; the cause of this deaxiation is the greatly lowered rate of development incident to the "period of quiescence." The next link in our chain of causes is the one that will account for the "period of quiescence." In his voluminous monograph on *Developmental Rate and Structural Expression*, Stockard (1921) reviews the armadillo situation and offers "an explanation of polyembryony in the armadillo," which involves

certain rather intangible morphological conceptions, such as "discontinuous mode of development," "developmental moments," "developmental arrests," and "alternation of generations." Stockard accepts my view that twinning in the armadillo and elsewhere is a result of an early retardation of development or "arrested development." He also adopts the budding hypothesis of Patterson, especially the latter's view that one embryo of each pair is a sort of accessory bud given off laterally from the primary bud.

Stockard credits me with having appreciated the significance of the "period of quiescence" in twin formation, but claims that I overlooked what he himself considers the very important fact that, during this period, the blastocyst lies free in the uterus. This, in spite of the fact that two references to this very fact were made on page 39 of my *Biology of Twins*. That there was no failure on my part to appreciate the physiological significance of the belated placentation is evidenced by the statement on page 88 to the effect that it is only after the physiological isolation of the quadruplet primordia that a nutritive connection is established between the embryonic vesicles and the maternal tissues. It seemed to me at that time almost an obvious inference from the facts that the "period of quiescence" and the failure to undergo placentation were physiologically related, but no categorical statement to that effect was made.

Stockard, therefore, is to be credited with emphasizing this point. Undoubtedly failure to undergo placentation at the time when eutherian mammals usually do is the immediate cause of the arrest of development. Evi-

dently the egg goes as far on its own resources as it can, and would, in any species of mammal, cease to develop if unable to get the necessary assistance furnished by placentation. What the nature of early placental aids to development are can readily be conjectured. They are primarily food and oxygen and a means of eliminating wastes. Doubtless, in the armadillo, the deprivation of all of these growth necessities brings growth to a nearly complete standstill. Patterson notes that cell division ceases but that the vesicle continues to expand through the excretion of liquid within the cavity of the vesicle. Protoplasmic activity must be going on all this time, but cell respiration must be largely anaerobic. Probably there is an accumulation of carbon dioxide and other metabolic wastes. This of itself would tend to check development. Stockard claims that the primary cause of the developmental arrest is lack of oxygen, and this may well be partially true. The real cause, however, is failure to attain at the proper time the essential growth stimulus which is normally supplied by placentation.

When we have added as a link to our chain of causes that of belated placentation, we still must account for this situation. Why does not the egg attain a placental connection when it first comes in contact with the maternal mucosa? This is a problem whose solution would get at the very foundations of the causes of twinning and might lead to an experimental control of twinning in mammals, including man. When blastocysts undergo placentation there is a mutual proliferation of tissues, both embryonic and maternal. Each seems to respond to stimuli given off by the other. If either

were non-sensitive no placentation would result. In this case, I believe, the non-sensitiveness is not of the embryo but of the maternal mucosa. It is slow to acquire the sensitivity that a uterus normally possesses in the presence of a blastocyst. Recent experimental work has tended to show that the sequence of events in mammalian gestation is controlled by an intricate system of hormones. The event of ovulation is quickly followed by the formation of a glandular body, the corpus luteum, which appears to control by its secretions further ovulation and to excite the uterine mucosa to co-operate with the blastocyst in placentation. If, for any reason, the functioning period of the corpus luteum should be delayed there would inevitably follow a delay in placentation. In my opinion this is a point of experimental attack. If my assumption be correct, early removal of the corpus luteum might be expected to inhibit placentation. In the armadillo we probably have a case of sluggish activity of the corpus luteum; for it grows very large and becomes very active at a later period, and excellent placentation finally results. Even if it should prove to be true that slow growth and sluggish activity of the corpus luteum is a part of our causal chain we would still have to discover the cause of this. The search for causes is endless.

The essentials in the causal chain of twinning in the armadillo, according to the foregoing theory, are as follows: (*a*) slow development or sluggish functioning of the corpus luteum; (*b*) failure of the maternal mucosa to respond to the presence of the blastocyst; (*c*) belated placentation; (*d*) cessation of development for about three weeks; (*e*) partial loss of polarity or deaxiation

of the ectodermic vesicle; (*f*) the physiological isolation of two, then four, growing regions on the blastoderm; (*g*) the independent development from the four growing-points of four complete embryos.

Almost equally important for an understanding of our problem is the very obvious renewal of developmental activity as soon as placentation is attained. The embryo is first aroused only locally, in the region of the "Träger," but, with the renewed vigor acquired through the establishment of this first placental connection, the wave of renewed growth energy sweeps distally through the tissues and ultimately strikes the ectodermic vesicle. This region of the embryo has been the most profoundly inhibited, partly owing to its inclosed position and partly because the ectoderm, and particularly the nervous ectoderm, is the most susceptible region of the embryo to growth-depressing agencies. It is this vesicle in which we get our first visible evidences of twinning, though it is my belief that the true physiological isolation of the quadruplet primordia considerably antedates their visible or morphological isolation. Once this isolation is accomplished twinning has occurred.

To summarize, then, there is a fairly general agreement among those of us who are interested in the causes of twinning that, in the armadillo, the fundamental cause is interrupted or retarded development at a critical period, followed by an isolation of four growing-points. The exact mechanism of isolating the four growing-points is still undetermined. Two theories prevail at present: the budding theory of Patterson and of Stockard, and the fission theory of which I am an advocate.

THE FISSION THEORY VERSUS THE BUDDING THEORY OF TWINNING IN THE ARMADILLO

At the present time two writers, Patterson and Stockard, hold to the budding theory, while Assheton and the writer feel that the process is not one of budding but of fission. Assheton says: "One cannot have budding unless there is a stock from which budding takes place. There is nothing in *Tatusia* (*Dasypus*) one can call a stock. The phenomenon is clearly that of fission." To this Stockard replies: "The use of the word bud or budding in connection with double embryo-formations as employed by Patterson (1914) has been criticized by Assheton, who suggests fission as the better word for the process. Such a discussion seems devoid of value and I employ the word bud to mean what is indicated above."

It is evident from this expression of Stockard's that he regards the distinction between budding and fission as a valueless splitting of hairs. I feel quite the opposite. To me the interpretation of armadillo twinning as a budding process is extremely misleading and involves a total misapprehension of the significance of the nature of twinning. In order that we may be on solid ground in this discussion it becomes necessary to re-examine in detail the actual facts upon which the theories of twinning are based. We are indebted to Patterson for his excellent description of the facts. It is only his interpretations that seem to me to be incorrect.

The armadillo blastocyst, just before it shows morphological evidences of twinning, is represented in Figure 46. It is now connected with the maternal mucosa by means of the Träger ring. Cell division has

been resumed and it is high time that an embryonic axis developed. There is, however, no clearly defined head process or primitive streak. The ectodermic vesicle,

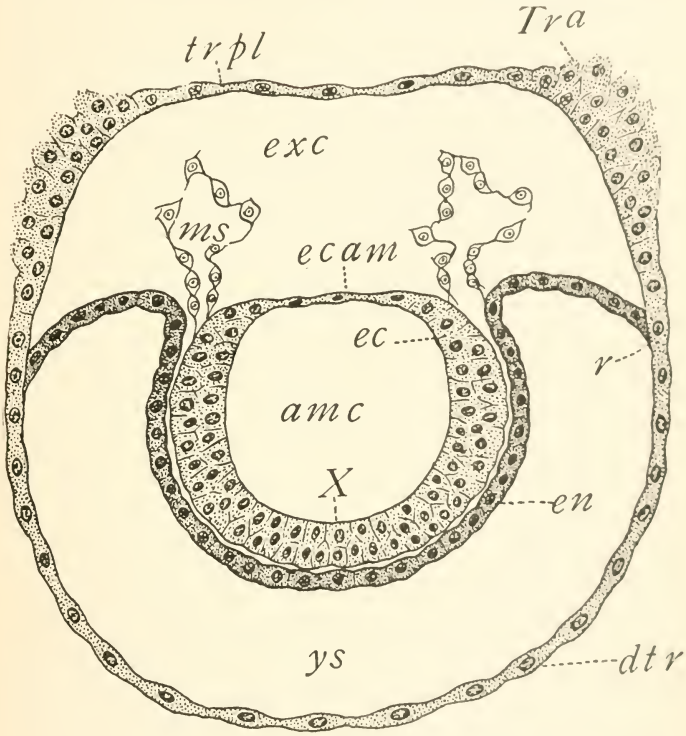


FIG. 46.—Section of an armadillo embryo, cut at right angles to the sagittal plane, showing the first evidences of the prospective twinning process. A secondary bilaterality has begun to be imposed upon the embryo by the bilaterality of the uterus. The point *X* is the original apical end of the single embryo. The two sides of the ectodermic vesicle (*ec*) are thicker than the point *X* and are the blastoderms of the twins. *Tra*, Träger or primitive placenta; *en*, endoderm; *tr pl*, trophoderm plate; *ec am*, ectodermal layer of amnion; *am c*, amniotic cavity; *ms*, mesoderm; *ys*, yolk sac; *ex c* extra-embryonic cavity. (From Newman after Patterson.)

which must be thought of as homologous with the medullary plate rolled up into a hollow ball, has thinned out in the roof to form the ectodermal layer of the amnion. The center of the floor is the region where we would expect to see the first sign of a differentiation of the apical point of the new axis; but this part is even a little thinner than are the lateral walls, directly to the right and to the left of the original apical region. The only evidence of a definite bilaterality in the ectodermic vesicle is seen in the fact that it is broader in one plane than in any other. The figure is drawn in this plane and shows in the mesoderm further evidences either that bilaterality has persisted in the vesicle or else that it has been established *de novo* as a result of the position of the vesicle with respect to the axis of the uterus; for the bilateral axis of the embryo coincides with that of the uterus even at this early period. The mesoderm begins to proliferate from two points where the ectoderm and endoderm part company. For some time the mesoderm consists of a considerable number of isolated thin-walled vesicles, but there is always a period when these small vesicles break together into two large vesicles, separated by a median mesentery that coincides with the principal axis of the untwinned embryo. Concerning this point it is important to note Patterson's comment:

> The earliest observed evidence which could be interpreted as representing the beginning of multiple embryos comes in the formation of the mesothelium—not in the manner in which the elements of this layer arise, for localized centers of proliferation were not found, but in the early formation of two large mesodermal vesicles through the fusion of smaller ones. The development of two mesodermal vesicles would not in itself be so significant, as it

might be *merely an expression of a bilateral arrangement of mesoderm similar to that of many other vertebrate embryos* [italics mine], were it not for the fact that they hold a position corresponding exactly to the two primary ectodermal buds; that is, they lie on the sides of the vesicle which are directed toward the openings of the Fallopian tubes.

This important statement seems to me to settle at least two questions that have puzzled us for some time. The first is that we have revealed to us the origin of the exact correspondence existing between the two pairs of twins and the two halves of the uterus. It will be recalled that there are always two fetuses attached to a right-hand placental disk and two more to the left-hand disk. These are natural twin pairs and show many evidences of an extremely close relationship. It is almost impossible to conceive of the vesicle as a bilateral organism coming to lie in such a fashion as to have its plane of symmetry coincide with that of the uterus. The alternative view, and one that agrees well with the facts, is that, either after its long period of quiescence the vesicle has lost any bilaterality that it may have possessed, or that up to that time it had never developed an axis of symmetry. Only the axis of polarity had been established and this had been nearly obliterated. The crosslike placental area of the uterus (see *The Biology of Twins*, p. 31) is very precise in its topographic outlines and it seems clear that the vesicle soon comes to be influenced by its location in such a way that a new symmetry system arises in response to the symmetrical conditions of the uterine environment. That the environment does, in certain cases at least, determine the axial and symmetrical relations of developing organisms has been repeatedly demonstrated by various authors, and

that this is only another instance of a very general situation seems evident. Much of the difficulty formerly associated with the fact that the symmetrical relations of armadillo quadruplets coincide with those of the mother thus disappears when we view the fetal symmetry as determined *de novo* by that of the uterus. The second point of importance that is brought out by Patterson's description inheres in the italicized words, for I believe that the bilateral arrangement prior to twinning which he points out is not at all the bilaterality of the untwinned embryo, but merely the result of a physiological isolation of two halves of the vesicle. It is the equivalent of what happens in a starfish blastula when two invagination areas become physiologically isolated so as to lie in equivalent positions to each other, so that each faces the other like a pair of mirror-images. The separation of the two gastrulation areas is obviously a sort of migration of cells toward the right and left of the uterus, leaving a thinned-out region in the middle line. This is more like a fission process than a budding process, for of the two embryonic areas it would be impossible to say which is the original individual and which is the bud. The concept of budding implies that the original apical point retains its identity and that the bud is a secondary, more or less lateral, new growing-point that has escaped from the dominance of the original or primary growing-point and has asserted its own independence. This is evidently Patterson's idea of budding, at least in so far as the formation of secondary buds is concerned, as will be made clear from the following quotation:

The primary buds do not develop for some time after the completion of the ectodermal vesicle, although their appearance

is anticipated soon after this period by certain easily detectable changes in the walls of the vesicle. It will be recalled that immediately after the ectodermal sphere has become transformed into a vesicle, that portion of the wall of the vesicle which is turned toward the free pole of the blastocyst is of a relatively uniform thickness. Very shortly thereafter one can detect a tendency in this region of the wall to become less thick. The thinning out may be due in part to an increase in size of the vesicle by the accumulation of fluid within its cavity, but undoubtedly in the main it is brought about through the *shifting of cells from here to the lateral portions of the wall*, for these show an increase in thickness.

The shifting of cells from the pole of the vesicle results in the formation of a thickened zone adjoining the thin or endothelial-like portion of the ectodermal vesicle. The zone is not uniformly thick, but is thickest at the two regions corresponding respectively to the right and left sides of the vesicle. One can therefore correctly speak of these thickened areas as lateral plates.

The primary buds arise from these lateral plates, and appear as two broad, blunt processes protruding from the sides of the ectodermal vesicle. Each bud involves the greater portion of the side of the vesicle, covering an arc of approximately 80 degrees on the circumference [Fig. 47, p. 114].

What better description of a *fission* process could one ask for than this? The pre-twinning stage of the vesicle, which it must be remembered has undergone germ-layer inversion so that the cells originally apical in position are now occupying the distal pole of the ectodermic vesicle, is characterized by the fact that the polar area is thickest. This was the prospective locus of the head of the untwinned embryo. Then this apical area becomes relatively thinner down the middle and two bilaterally arranged, thickened areas arise and are distinctly separated by the median, thinned-out area. Is this budding or fission? The so-called primary buds

are merely the bulges due to the presence of the two thickened areas, the medullary plates of the now twin embryos. This is the true twin stage in armadillo development. Before any further differentiation occurs, however, these twin embryonic areas once more undergo bilateral fission, rather less complete than the first fission, for the median area does not, for a time at least,

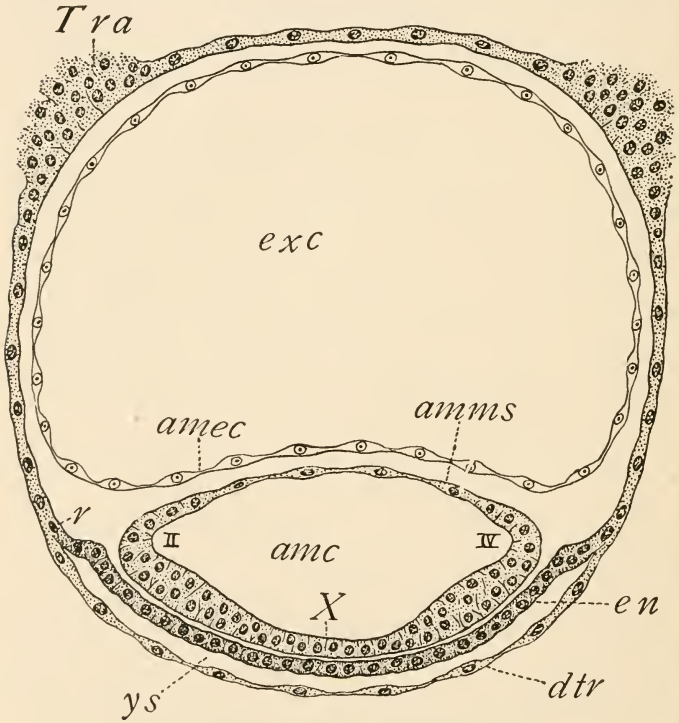


FIG. 47.—An armadillo embryo in the true twin stage. The thickened plates of ectoderm below the figures II and IV are the embryonic primordia of the twin embryos and are as yet undivided to form the quadruplet condition. Lettering same as in Fig. 46. (From Newman after Patterson.)

thin out so markedly as when the first twinning process occurred. The twinned embryonic areas, one on each side of the vesicle, do not completely separate but remain united by a relatively thick band of ectoderm and it is the gradual severing of this connection, *beginning at the posterior end*, that Patterson has mistakenly interpreted as a secondary budding process. His own account shows this:

The formation of the secondary buds immediately follows the establishment of the primary diverticula. . . . . Each primary bud gives rise to two secondary buds, and consequently there are four secondary diverticula. Each secondary bud carries the rudiment or primordium of an embryo. The first step leading to the development of the secondary diverticula consists in the formation of two thickenings in the wall of each primary bud. One of these areas lies at the tip of the bud, while the other appears slightly to the left (as viewed from above) of the tip. The secondary buds then arise from these areas as blind diverticula, which extend down along the inner surface of the yolk-sac entoderm.

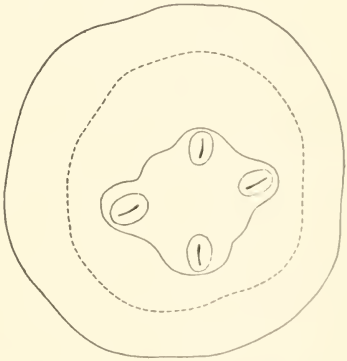


FIG. 48.—Outline polar view of an armadillo quadruplet egg after the completion of the process of twinning. The four embryonic areas are entirely separate; each has established its own axis and is about to migrate backward toward the placental region. The head ends of the four embryos point toward the center of the vesicle. The posterior ends are beginning to push outward and give rise to the so-called "buds" of Patterson. (After Patterson.)

Let us look for a moment at Patterson's figure illustrating the four "buds" (Fig. 48). The

first thing of importance that I note is what Patterson has apparently forgotten: that the apical or head ends of the four embryos are all directed inward toward the center of the vesicle. The heads are already widely separated as are also the embryonic areas. The "buds" are merely outpushings at the posterior ends of the embryos involving largely extra-embryonic (amniotic) ectoderm. It should be entirely obvious from Patterson's own account that the essential acts of twinning are entirely finished before this "budding" process begins. What then are these so-called "buds"?

This question may be readily answered after studying the typical embryonic history of non-twinning armadillos, for it is here that the clue to their interpretation exists. According to Fernandez (1914), who has studied the development of the non-twinning species *Euphractus villosus*, the medullary plate of the single embryo arises from the distal thickening of the ectodermic vesicle, just as it begins to do in our twinning species. Instead, however, of remaining at the distal pole, farthest from the placenta, the embryo grows backward out of the amnion, which remains attached to the distal endoderm. It leaves the amnion by means of a process, the "bud" of Patterson. Not only does the posterior end grow backward but the head as well moves along the inner wall of the ectoderm carrying attached to it an amniotic connecting canal that remains as a hollow string connecting the amnion of the embryo to the rudiment of the original amnion at the opposite pole. It is evident, therefore, that Patterson's "secondary buds" are merely the beginnings of amniotic outpouchings which are destined to act as migration canals through which the

individual embryos may pass in order to reach the opposite end of the vesicle, where lies the Träger. Each embryo in our twinning species, in thus migrating to the placental pole of the vesicle, behaves just as if it were the only embryo in the vesicle. It seems clear then that, unless we are prepared to call the outpouching of the non-twinned embryo of *Euphractus* a "bud," the term "bud" for the homologous structure of *Dasypus* is entirely a misnomer. It seems strange also to think of embryonic primordia budding at the tail end, as they would be doing if this is budding; for in typical cases of budding it is implied that the budding region is a new apical region or head region. It is quite certain that in all of Stockard's work he considers his "buds" as new head regions.

When the embryos first begin their backward migration toward the Träger, the paired embryos of the right side remain broadly attached by a band of ectoderm. The same is true of the pair on the left side. They therefore migrate together for a short distance and are in the same amniotic canal. Soon, however, the connecting band thins out and breaks apart in forklike fashion, and the two embryos proceed to grow and migrate down two distinct canals, tail first, as though switched backward onto diverging tracks. There is left, therefore, for a little distance from the common amniotic vesicle, a common canal which soon splits into the two individual connecting canals. One can always identify the twin products of one side of the vesicle by the fact that their individual connecting canals thus unite before entering the common amnion. It is this forking apart of the posterior ends of the embryos that gives the appearance

of budding. Patterson has noted that buds II and IV seem to occupy the original site of the "primary buds," that is, respectively toward the right and toward the left, while buds I and III appear to come off slightly behind and to the left of, respectively, buds II and IV. He is therefore inclined to consider buds II and IV as continuations of the original twin primordia, the "primary buds," and that I and III are lateral or accessory buds. In support of this view it is not infrequently noted that I and III are usually not quite so advanced as are II and IV. The lateral budding notion can hardly continue to be valid in view of what has already been stated, but it is true that the position of one of each pair remains lateral and the other lies to its left. There is evidently some very accurately balanced behavior here, whatever its significance. The result is that each embryo comes to occupy its full quadrant of the vesicle. What appears to happen is that one of the migrating embryos keeps the original direction while the other is shunted off at a considerable angle. Why in both cases the shunted-off individual goes to the left is a question that at present I am entirely unable to explain. A careful re-examination of my own material and of some of Patterson's figures leaves it an open question in my mind whether the right embryo of each pair always retains the original direction of growth and the left is caused to diverge. There are certainly some cases in my possession where the point of attachment of the placenta is more nearly lateral in the left-hand embryo than in the right and there are undoubtedly many instances in which the left-hand embryo of a pair is more advanced in development than

is the right. It would be interesting to know just how general is the condition which Patterson describes.

The preceding interpretation of twinning disposes, I believe, of the lateral budding idea of Patterson. Certain evidences favoring the fission theory have been presented in passing, but the main evidences that twinning in the armadillo is a case of longitudinal or bilateral fission inhere in the facts brought out fully by the writer several years ago in connection with closeness of resemblance and with symmetry reversal. It was shown quite conclusively that the resemblances between these twins are as close as are the right and left sides of a single individual, very closely approximating complete identity. It was shown, in addition, that asymmetrical peculiarities occurring on one side of one twin were very frequently found on the opposite side of the other twin. If there is any validity at all to this idea of symmetry reversal it is obvious that it must have significance as the residue of a former bilateral symmetry of the undivided embryonic axis, and it is equally obvious that the only kind of division that would preserve such an original symmetry and show it in mirror-imaging is the method of bilateral fission. Lateral budding could never have any such results.

Stockard's theory of twinning in the armadillo is based on Patterson's budding theory, and is therefore open to the same objections. A few quotations from his principal paper will reveal this point of view:

> The double primitive streaks in the hen's egg and other forms all lend themselves to strengthen the interpretation that double embryo-formation first asserts itself by a double gastrulation or blastopore formation, which is initially a process of double

instead of single bud formation. Patterson's description of the origin of the quadruplet buds in the Texas armadillo furnishes the most striking case in the study of these conditions. And we may conclude that the budding or *accessory embryo-formation* in the egg of the armadillo is exactly the same developmental process as that which gives rise to twins and double individuals in other vertebrate eggs.

In another place he says: "There is reason to believe that, aside from the external factors discussed, the armadillo egg is highly disposed toward the *formation of accessory embryonic buds.*" Again, in attempting to explain why the deer, although it has a "period of quiescence," fails to produce twins, he says: "The egg of the deer may possess only a very slight tendency toward *accessory embryo formations.*"

Exactly what does Stockard have in mind when he uses the term *accessory bud?* It is clear that he uses the term advisedly and means to imply just this: that the original embryo retains its identity, but that, through its loss of dominance over the rest of the blastoderm, accessory or secondary buds arise which give rise to additional embryos. Evidently this is part and parcel of the theory of budding, for a bud is essentially an offshoot of a previously existing individual or of a common stock. If, therefore, the phenomenon of twinning in the armadillo turns out not to be budding at all, but fission, the whole budding theory, together with the causal theories based upon it, falls to the ground.

CHAPTER IX

THE MODES AND CAUSES OF HUMAN TWINNING

INTRODUCTION

We recognize two types of twinning in man—one-egg twinning and two-egg twinning. In our present discussion we shall limit ourselves to one-egg twinning; for two-egg twinning is, strictly speaking, not true twinning at all.

It has now come to be very generally agreed that separate one-egg twins (duplicate or identical twins) belong to the same series and result from the same causes as conjoined twins or double monsters. If this conclusion is valid, acceptable theories of the modes and causes of twinning must be in conformity with conditions in both types of twins. It is my belief that the clue to the mode of human twinning must come from a study of the various incomplete stages of twinning exhibited by conjoined twins.

THE MODE OF ORIGIN OF CONJOINED TWINS

For a long time it has been tacitly assumed, and rightly so, that conjoined twins are the products of some kind of division of a single egg. The grounds for this assumption are: (*a*) they are always of the same sex; (*b*) they very frequently show *situs inversus viscerum*; (*c*) they are usually cosmobia, i.e., they are joined in symmetrical positions with regard to one another, and homologous parts of the two systems are always united.

Conjoined twins are united in a great variety of ways. By far the commonest condition is that in which the anterior parts are separate and the posterior parts are fused. There are, however, rare cases of twins, called Janus monsters in which the heads are less completely separate than are some of the more posterior parts of the body. It is not fair to say that these individuals have a single head and two complete bodies, for even the head is double in that there are two faces.

When twins are united they are usually connected by ventral regions of the body though they may later come to lie in such a way that they fail to show this condition clearly. There is no certain case in which the dorsal parts of the twins remain single while the ventral parts are double. There is nearly always dorsal duplicity and ventral unity. Even in the cases of so-called pygopagi, in which the twins seem to be joined back to back, the conjoined organs are not the vertebral column nor the central nervous system, but are nearly always certain ventral structures such as the intestine or the urethra. Rare cases occur in which otherwise complete twins are lightly united in the head region, as for example in the region of the forehead or the top of the head.

If, instead of attempting to classify and to interpret these double monsters on the basis of the degree of more or less mechanical union which they exhibit, we give attention to the degree of duplicity of the various parts of the body and of its organs, the problem becomes much simpler.

Are conjoined twins to be viewed as incompletely fused separate individuals or as incompletely divided

single individuals? The decision between these alternatives is crucial for our theory.

Granted that they are derived from a single egg, do the two parts of such twins come from two independently arising embryonic axes which subsequently come to fuse together in certain regions and remain separate in others; or do they arise as the result of a more or less complete separation of the bilateral halves of a single embryonic axis? The first alternative, which involves the idea of fusion of separate embryos, meets with an almost insuperable obstacle on account of "the complete bilateral symmetry of the two components in true double monsters (diplopagi), since there is no force to oversee and adjust the two components in the exact relationship necessary for this result" (Wilder, 1904). There may be, however, a minor degree of purely external or mechanical fusion due to previously separated parts remaining too closely approximated. Such embryos may be pushed or crowded together, for they lie within a single amnion and have no means of avoiding contacts. For enlightenment as to the mode of origin of human double monsters we may profitably turn to the much better understood conditions in the birds; for we have reason to believe that mammalian conditions are quite similar in most respects to those of reptiles and birds. There were, it will be recalled, two types of avian double monsters in which there was more or less unity of the head regions and duplicity of the trunk region. The first of these types was explained on the basis of the existence of two more or less separate bilateral growing regions, the head process and the primitive streak. Either region may undergo complete or partial twinning without the co-

operation of the other. The second type of double monster was due to the mechanical head-on collision of two embryonic axes as in Figure 37. So far as I am aware there are no cases of human double monstrosity that are to be explained as due to crude mechanical fusions of the latter sort; hence it would appear that all cosmobia are to be explained as products of fission or physiological isolation of the bilateral primordia. Janus monsters, with their two faces and with brains partially double and partially fused, but with bodies more completely double (see *The Biology of Twins*, Fig. 2, *b*), are to be viewed as instances of the fission of the head process and of the primitive streak in which the latter was more complete than the former. The cyclopian monster shown in *a* of the same figure is probably another illustration of the independence of the two twinning regions. The bodies are completely isolated except for external fusions, while the head is not double at all, but just the opposite in that even normally paired structures such as the eyes are single. The same factor that produced cyclopia in the head region has evidently produced twinning in the secondary growing region, the primitive streak. This is rather a striking confirmation of the theory that twinning and single monstrosities are due to the same cause—retarded development. A very large percentage of human double monsters are classed as anadidymi, in which the anterior parts are more double than the posterior parts. This was even more strikingly the case for fish double monsters. The anadidymi represent the standard type of vertebrate double monstrosity and it is toward their explanation that most of the theories of the past have been directed.

THEORIES OF THE MODE OF ORIGIN OF HUMAN DOUBLE MONSTERS

In reviewing Dareste's work on the origin of double monsters in the chick we had occasion to present a brief history of European opinion as to mode of origin of human double monsters (see pp. 74–76). These views need not be restated here. Suffice it to say that the weight of opinion was in favor of an origin by fusion of separate embryos. In America, however, we find an early expression of the fission theory. In 1866 G. H. Fisher postulated a theory that human double monstrosity is due to an early total fission of the embryo, followed by a subsequent fusion of the two parts. He says that double monsters "are invariably the product of a single ovum, with a single vitellus and vitelline membrane, upon which a double cicatricula, or two primitive traces are developed. The several forms of double malformation, the degree of duplicity, the character and extent of the fusion, all result from the proximity and relative positions of the neural axes of the two more or less definite primitive traces developed on the vitelline membrane of a single ovum." This idea, the reader will note, implies that all united parts of double monsters are fusion products, a view quite inadmissible in view of the various facts already stated and that are soon to be discussed. The fission idea is also far from clear. We are not told when or how the two primitive traces originate. Fisher uses the word "fission" loosely to mean some sort of dividing process giving rise to two embryonic areas on one egg. He has no conception that even remotely resembles that involved in our theory of fission which has been several times stated.

In 1904 Wilder in an important discussion of human duplicate twins and double monsters proposes the "blastotomy theory" of such duplicities. His idea, which has already been discussed in *The Biology of Twins* and need only be mentioned here, is that separate twins result from the complete separation of the blastomeres of the two-cell stage of the ovum, and that double monsters result from incomplete separation of these blastomeres. The degree and position of the union between these twins are attributed to variations in the points of contact of the two cells. If they remain attached by apical ends, we would have Janus monsters; if by the basal ends we would have pygopagi; if by ventral sides, thoracopagi. Since the discovery of the mode of twinning in the armadillos Wilder himself has abandoned his view in favor of the "budding theory." If, however, the budding theory turns out to be inadequate for the armadillos, there is even less reason for its adoption in the case of human one-egg twins, and especially is it inapplicable to that of conjoined twins.

STREETER'S THEORY OF THE ORIGIN OF HUMAN TWINS

In view of the fact that no really early cases of one-egg twinning are known for man, the theory has prevailed that the process must be closely similar to that of the armadillo. The facts that in both man and the armadillo the uterus is simplex, that there is a similar ectodermic mass and subsequently a similar ectodermic vesicle involving a similar method of amnion formation, have made it seem highly probable that twinning in man is equivalent to the first step in twinning in the armadillo

which Patterson has called "primary budding," but which I interpret as a very simple sort of bilateral fission determined by the bilaterality of the uterus. If we could only secure an early normal stage in man equivalent to the first fission stage in the armadillo we could readily settle the question as to whether the same mode of one-egg twinning occurs in these two quite different mammals. G. L. Streeter (1919) has recently made an exhaustive study of a very early human one-egg twin embryo (the Mateer ovum) which he thinks throws considerable light on the question before us. This is much the earliest stage of human twinning we have discovered and deserves our careful consideration.

The twin embryos are markedly different in size and in stage of development. The larger one (which Streeter calls the primary embryo) apparently lies in normal relation to the yolk sac and placenta (Fig. 49, C, p. 128). "It is in the presomite stage and has only just acquired a primitive groove." The smaller (which Streeter calls the twin embryo) is in a stage about equivalent to that of the armadillo just prior to the first step of twinning or before the embryonic axis is definitely established. This smaller embryo is so abnormally situated with reference to the larger embryo and to the placenta that it probably never could have attained satisfactory nutritive relations. It does not seem likely, therefore, that we have in this case a typical instance of twinning such as might produce duplicate twins. In addition to the fact that the smaller twin is so obviously abnormal, the twins differ in other ways from what must be the normal situation in duplicate twins. Streeter's twins have entirely separate amnia, and if the small twin were to placentate it would have a

separate placenta. This is in contrast with the fact that some human twins, especially double monsters, have a common amnion; and one-egg twins always have a common discoid placenta.

On the basis of his studies of this embryo, however, Streeter proposes a theory of human twinning which is

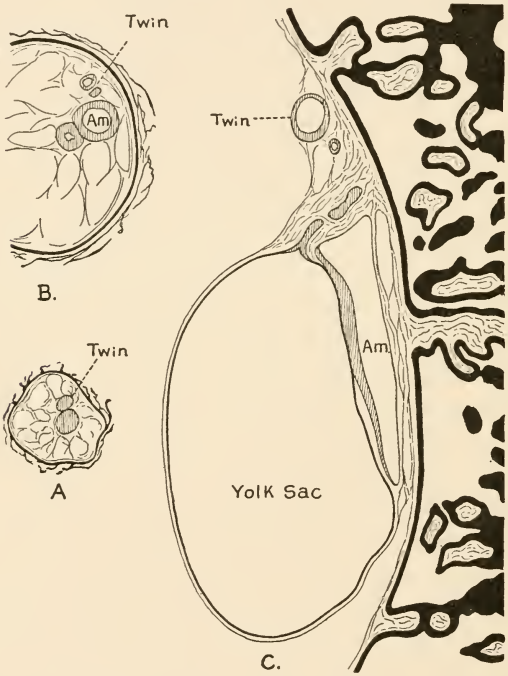


FIG. 49.—Schematic drawing, showing Streeter's idea of the formation of a human one-egg twin. The stages are drawn to the same scale of enlargement so that they may be directly compared. A. Stage corresponding to the Miller specimen, showing a hypothetical twin budding off from the primary embryonic node. B. Stage corresponding to the Bryce-Teacher specimen. C. The Mateer specimen. The relatively small size of the twin in this specimen, and the detachment of the yolk sac from the amniotic vesicle are indications of arrest in development. (From Streeter.)

essentially a fission theory similar to that proposed by Assheton for his early sheep twins. Streeter considers that the fission process takes place at the inner-cell-mass stage. This mass or "embryonic node" is believed to undergo subdivision into two more or less equivalent masses. If the two masses are equal in size

then chances of developing in an orderly manner would be equal, and this is presumably what happens in most instances of identical twins. Where the secondary bud is merely a fragment of the original mass we would expect that there would be some degree of differentiation; but the process of development would soon be arrested, and at term the stunted bud would be found as a small epithelial cyst on the placenta near the attachment of the umbilical cord. In case the twin-bud is only partially detached from the primary node there would exist the basis for the various types of double monsters and teratoma.

It will be noted that Streeter uses the language of the budding theory, probably influenced by myself and by Patterson, but the process which he describes and figures cannot rightly be called budding, especially if the embryonic node divides into two equal masses, for in this case we could hardly speak of one as the bud and the other as the stock. As has already been said, the theory in no way resembles the budding theory of Patterson, but is definitely a fission theory. The chief objections to Streeter's theory are that it fails to account for the symmetry relations of duplicate twins and for the fact that such twins frequently have a common amnion. Moreover double monsters, which are believed to belong to the same series of twins, always have a common amnion and have strikingly symmetrical and intimate interrelations: conditions that could not be accounted for unless the embryonic axis were either

formed or were forming during the twinning process. The type of twinning described by Streeter takes place at an early blastula stage and should be classed as a case of fission of an early blastoderm to form two separate embryonic primordia.

AREY'S THEORY OF THE ORIGIN OF HUMAN TWINS

Just as I was revising the manuscript of this volume there appeared two papers by Arey (1922*a*, 1922*b*), describing certain early human one-egg twins, that throw new light on our present problem. It is not uncommon to find in human beings cases of tubal pregnancy. One or more ova are fertilized in a Fallopian tube and because of certain pathological conditions remain in the tube and acquire a sort of makeshift placentation. Embryonic development may proceed for months before fetal death occurs. Arey has brought together, after a rigid examination of the literature, some sixty cases of human tubal twins, about two-thirds of which are monochorial. In view of the fact that uterine monochorial twins (probably always one-egg twins) are only about one-fourth as numerous as dichorial twins, it appears that tubal monochorial twins are eight times as frequent as "might be expected if the tube were no more favorable than the uterus as a seat for twin production."

Arey himself describes two cases of monochorial twins that are especially significant:

> The first of the two specimens consisted of a single chorionic sac which contained twin embryos, each 12.3 mm. long. There is a common yolk sac from which distinct yolk stalks arise near together and pass to their respective umbilical cords. . . . . The second new twin specimen is in some respects more interesting. Within a single chorion were twin embryos of 11.5 and

12 mm. Each had its individual umbilical cord; these were attached to the chorionic wall, a quadrant's distance apart. Adherent to the amnion of one embryo was a yolk sac of normal size. . . . . The other embryo has no yolk sac.

Arey enters into a discussion as to the bearings of the lack of yolk sac in the latter twin, which seems to be of little value for our theory. Our chief concern has to do with the mode of origin of these two cases of human twins. The first case is almost certainly a case of double gastrulation of a distinctly symmetrical sort like those of the starfish shown in Figures 4–6 or like certain chick twins such as that in Figure 36. The mode of origin of the other twin embryo is uncertain. Since the two embryos are not attached to the same yolk sac they have probably originated from an early total fission of the blastoderm or embryonic node much like the hypothetical case of Streeter except that the fission must have resulted in two practically equal primordia both of which were able to form a placenta.

MODES OF HUMAN ONE-EGG TWINNING

Although the evidence is still somewhat meager we are now in a position to state with some confidence that the same three modes of one-egg twinning occur in the case of man as have been described for previous invertebrate and vertebrate types: (*a*) twins produced by fission of the blastoderm, as illustrated by Streeter's case and the second case of Arey; (*b*) twins produced by double gastrulation, as in Arey's first case; (*c*) double monsters, and possibly some entirely separate twins, produced by partial or complete fission of the bilateral halves of a single embryonic axis.

THE CAUSES OF TWINNING IN MAN

The most nearly direct evidence bearing on the cause of monozygotic twinning in man is derived from certain data presented by Arey (1922*b*), already referred to above. He has shown that monochorial twins are many times as numerous in the Fallopian tubes as in the uterus. The tubes are far from being a normal locality for the placentation of the embryo and there is reason to believe that even the makeshift placentation that does take place is greatly belated. If this assumption be warranted we have a situation quite similar to the "period of quiescence" in the armadillo, and the consequence would be the same: partial loss of axiate organization and a physiological isolation of two secondary apical points or points of gastrulation.

Thus we might be able to account for monochorial twinning in the Fallopian tubes, but we would still have to explain uterine monochorial twinning. The nearest approach to direct evidence of the causes of uterine monochorial twinning is furnished by Stockard (1921). A case of triplets came to his attention in which one individual was born a normal female baby.

> After delivering the child the physician, Dr. Erdwurm, noted that a second chorionic sac ruptured and discharged its fluid. Later two dead twin female fetuses were delivered. These lay in a common amnion with their umbilical cords twisted around one another in such a way that they had probably cut off both blood connections.

In further comment on this case, Stockard goes on to say:

> My interpretation of this triplet condition is as follows: The mother liberated from the ovary two eggs, both of which became fertilized and began development. One became implanted

slightly before the other and developed into the single living girl. The second egg was not so favorably implanted as the first; this is indicated in the specimen by the lower placenta riding upon the larger one. The delay in implantation, due to the presence of the first egg, caused a slow rate of development at an early stage in the second and two embryonic buds arose instead of one, just as was described on the germ ring of the fish. In this human specimen there is fortunately present the physical cause that might have produced the delay.

This explanation of Stockard's has unfortunately a very limited application, for it is extremely rare that one-egg twins occur along with another embryo. As a rule the egg destined to produce twins has the whole uterus to itself; it could not be retarded by the prior placentation of another egg. We must therefore look elsewhere for probable retarding agencies. Three possible retarding factors seem possible:

1. *Understimulation of the egg, due to some defect in the development-initiating mechanism of the sperm.* Davenport has shown that twinning is rather strongly inherited in the male line. If this be the case it could hardly affect two-egg twinning, since this is a phenomenon of ovulation and concerns only the female. It would seem then that only one-egg twinning could be affected through the male line. If the egg were retarded through insufficient stimulation on the part of the sperm it would probably undergo belated fission, the consequences of which would depend upon the degree of retardation.

2. *Belated placentation, due to a failure of the corpus luteum to stimulate the uterine mucosa.* This condition merely implies some physiological discoördination between the various intricately interdependent factors responsible for implantation of the ovum. The weakness

of this view is that the same mechanism would presumably persist throughout the reproductive life of a given mother and she should always produce twins. Such a condition, however, does not prevail, for almost without exception mothers of one-egg twins have also single children. It is barely possible, however, that cases of single offspring from parents exhibiting one-egg twinning are not true single offspring but that each is the survivor of a pair of twins, one of which has succumbed to the ever-present hazards that prevail especially in connection with one-egg twinning. This particular explanation is the one that was adopted for the armadillo case, where prenatal mortality is extremely low. On the whole this seems the least objectionable causal theory of twinning in man.

3. A third possibility is that *twinning is a hereditary* character dependent upon a recessive gene. The effect of this gene would have to be thought of as an unfavorable growth-retarding factor that causes a temporary "period of quiescence" like that in the armadillo, resulting in belated placentation and twinning. The cause of twinning, according to this theory, is purely intrinsic, unaffected by environment, and could be as readily transmitted through the sperm as through the egg. If two individuals heterozygous for the twinning gene mated, some of the zygotes would be homozygous for the character and twinning would result. This theory would account for the fact that in twinning families there may be some single offspring. This genetic theory of twinning seems to me on the whole somewhat fantastic, but it can hardly be excluded as one of the possibilities, especially in view of Davenport's discovery.

CHAPTER X

DEVELOPMENTAL HAZARDS OF HUMAN TWINS

SEPARATE TWINS

ON THE INFLUENCES WHICH TWINS, ESPECIALLY ONE-EGG TWINS, EXERT UPON EACH OTHER IN THE UTERUS

A popular impression prevails that in human twins one is usually stronger and more vigorous than the other. Observations of the writer and of others who have interested themselves in these matters tend to bear out this impression. Even in the case of so-called duplicate or identical twins, the products of a single egg, there is nearly always a more vigorous twin who is the dominant member of the combination. There is also a somewhat vaguely expressed feeling among families in which twinning has occurred that one twin has in some way drawn upon the vitality of the other or has inherited more than his fair share of certain essential qualities, leaving the other somewhat depleted in energy and vigor. A more definite form of this type of idea, to wit, that one twin is commonly sterile, has come to me several times of late. This idea may have had its origin in the freemartin situation among cattle, where a female calf born twin to a male is nearly always sterile. The possibility that human freemartins may occur has never been adequately affirmed nor denied.

It is my belief that these popular impressions are not without foundation. There is abundant evidence, especially in the case of one-egg twins, that, as the direct

result of the twinning relation, one twin tends to gain a physiological ascendancy over the other, to the slight or very great detriment of the latter. As to the extent to which one twin may harm the other during pregnancy there is considerable difference of opinion.

Spaeth (1860) was probably the earliest observer to study this problem. He was chiefly interested in the question whether the interinfluence between fetuses was greater in one-egg than in two-egg twins. His material consisted of sixty-five pairs of new-born twins and their embryonic membranes. Whether the twins were the products of a single egg or of two eggs was judged by the relations they bore to the placenta and the other membranes, especially the amnion. A comparison between one-egg and two-egg twins showed that twins of both kinds are nearly always rather markedly unequal in size and in body length. In only three cases out of the sixty-five examined were the twins even of similar size and length. One of these cases of striking similarity, judged by their possession of a common placenta, common chorion, and common amnion, was undoubtedly a case of one-egg twins. The other two cases of close similarity were in two-egg twins. There was no evidence that the twins of either type had any definite physiological effect upon each other and Spaeth concludes that, although twins are so closely associated during pregnancy, they maintained a high degree of independence. In only one respect does he see evidences of interinfluence: in the occurrence of *situs inversus viscerum.* In a number of cases he noticed in one of the twins a reversed symmetry of stomach, heart, liver, and other more or less asymmetrical organs. As the whole ques-

tion of symmetry in twins is discussed in a later chapter of this book, we may postpone for the present a statement of Spaeth's opinions on this subject.

Schatz, who has written more extensively than any other writer about human one-egg twins, holds quite a different opinion from that of Spaeth as to the influences of twins upon each other. This author had the advantage of an adequate mass of data: an admirable collection of twin embryos and fetuses, together with their fetal membranes, from the Marburg and Rostock gynecological clinics. No other body of data on human twins comparable to this has ever been brought together. In several extensive tables Schatz gives lengths and weights, together with percentage differences in weights and lengths of twins. These are put into groups based on the length and weight of the larger twin. Two classes of twins are distinguished:

A. Two-egg twins
   1. Twins in which the two placentae are entirely separate
   2. Twins in which the two placentae are more or less fused
B. One-egg twins (always with but one placenta)

The abundance of material enables the author to compare the developmental differences of the two classes of twins at various periods of pregnancy, instead of only after birth, as Spaeth had done. This method reveals the following important facts:

a) The differences between two-egg twins, irrespective of whether or not the placentae are separate or fused, increase steadily up to and after birth.

b) The differences between one-egg twins are greatest at about the middle of pregnancy and decrease steadily until or after birth.

c) The result is that the two types of twins show about equal differences at birth, a fact which is in agreement with Spaeth's findings.

d) At birth one-egg twins after a long period of decreasing difference, and two-egg twins after a still longer period of increasing difference, come to a period of approximate equality. It follows from this that during the whole period of pregnancy one-egg twins are distinctly more different than are two-egg twins. And this is a more striking circumstance in view of the fact that their origin from one egg should tend to make them more alike rather than more different.

e) The only conclusion to be derived from these facts is that the conditions of one-egg twinning tend to cause one twin to have a pronounced effect upon the development of the other. Schatz has made an exhaustive study of the ways in which one twin may influence the other.

THE DISADVANTAGES OF TWINNING

Before entering upon an account of the ways in which human one-egg twins influence each other's development, let us consider briefly some of the general disadvantages of twinning over single births. The human uterus is of the simplex or undivided type and is adapted for the really satisfactory gestation of but one fetus at a time. When two or more fetuses come to occupy the space usually filled by one, the twins, whether of the one-egg or two-egg type, crowd each other and compete for the common food supply. In the case of two-egg twins it probably often happens that one egg reaches the region of attachment first and tends to occupy the

available area to the complete or partial exclusion of the other. Stockard describes one case which he interprets in that way, in which the later egg, failing to gain a good placental attachment, underwent twinning, probably as the result of retarded development. Subsequently at about the middle of pregnancy the twin embryos died through a complete shutting off of nutrition, while the original single fetus went on to full term. It seems probable then that the main influences exercised by two-egg twins upon each other are the result of competition for placental surface. In addition to the extra hazards due to competition, twins seem to fall heir to all of the ordinary hazards met with by single fetuses, such as loosened placenta, twisted and knotted umbilical cord, stricture or breaking of umbilical blood vessels, rupture of amnion, loss of amniotic fluid, and the resultant adhesions. The period of uterine gestation is at best a hazardous one, but, quite in addition to all of the hazards that are met by single embryos and those that are shared also by two-egg twins, there are certain very serious special dangers that fall upon one-egg twins by reason of their close genetic relationship.

THE SPECIAL HAZARDS OF ONE-EGG TWINS

For the data herewith presented I am indebted to the numerous contributions to our knowledge of the developmental physiology of one-egg twins by Friedrich Schatz. These papers all appeared in the *Archiv für Gynaekologie* between the years 1882 and 1900. This author had the advantage of studying abundant material well preserved and adequately injected. No question seems to exist in his mind as to the occurrence of human one-egg

twins and there seems to be no difficulty in distinguishing one-egg from two-egg twins. A study of the detailed anatomy, especially the vascular anatomy of twin fetuses, together with that of the fully injected placentae and umbilical blood vessels, enables the author to determine the probable mechanism of the interinfluences of one-egg twins. Leaving out of consideration all injurious conditions which one-egg twins may have in common with two-egg twins or with single fetuses, let us focus our attention upon those interinfluences peculiar to one-egg twins.

Schatz distinguishes two types of fetal interinfluence incident to one-egg twinning. The first is associated with *situs inversus viscerum* or the possession by one of the twins of an asymmetry of the heart, stomach, and viscera which is the mirror-image of that of the other twin or of that characteristic of the species. This reversal is conceived of as a direct result of the twinning process, though there is no definite theory to account for it. All degrees of inverse symmetry are noted, ranging between a slight degree of it to complete reversal. It is very common in conjoined one-egg twins and relatively rare in separate one-egg twins. Schatz considers that inverse symmetry of the blood vessels, especially when the reversal is slight or incomplete, has very serious consequences for the unfortunate twin in which the inverse symmetry exists. Among other things, it may lead to a bad connection with the umbilical blood supply, which remains normal in its relations. Schatz enters into a detailed discussion of the secondary effects upon the vascular system of a fetus in which inverse symmetry exists and cites a number of cases of badly

deformed twins which are interpreted as the result of such an original inverse symmetry. It is interesting to note that symmetry reversal, besides being diagnosed as a definite criterion of one-egg twins, is considered as a hazard of twinning. Further discussion of reversed symmetry is to be found in chapter xii.

INTERINFLUENCES OF SEPARATE ONE-EGG TWINS

Human one-egg twins have a common discoid placenta to which are attached the two umbilical cords. As a rule the two cords are symmetrically placed upon the placenta, though there are some noteworthy cases of asymmetrical attachment. Sometimes the two cords are near together in the center of the placenta; sometimes they are on opposite sides and near the margin. There is even some evidence that the attachment of the two cords more or less closely coincides with the right and left sides of the uterus, reminding one of the situation in the armadillo. Whatever may be the point of attachment of the two cords on the single placental area, the twins divide this area more or less equally between them. There is opportunity for competition here; for the twins may develop at slightly different rates and the one that first develops a placenta is likely to acquire more than its fair share of placental area, and hence more than half of the available nutriment. This may account for a part of the marked size difference between one-egg twins during the middle period of pregnancy.

A far more important condition leading to interinfluence arises probably as a direct result of a competition for placental area. In the zone of competition the separate placental circulations of the twins come very

closely into contact and more or less extensive anastomoses of capillaries, arteries, and veins take place between the two circulations. Four types of vascular intercommunication are distinguished in these twin placentae:

A. In almost all one-egg twins there occur in the competitive zone twenty or more villous trees which are occupied in common by the circulations of the twins. The arteries of one twin occupy half of such a villous tree and the veins of the other twin occupy the other half. The real connection between the circulations here is through capillaries only.

B. In addition to the villous transfusion there may exist cases with one or more superficial arterial anastomoses.

C. Instead of arterial, there may be one or more venous anastomoses.

D. Many placentae show, in addition to villous transfusion, both venous and arterial anastomoses.

Types *A* and *C* are rare; types *B* and *D* are frequent. In brief, there are nearly always at least superficial arterial anastomoses, either with or without compensating venous anastomoses. This region of intercommunication between the placental vascular systems constitutes what Schatz calls *the third circulation*. This third circulation has a volume only about one-tenth or even one-twentieth as great as that of the general circulation of one twin. Small in volume as this may be, much of the welfare of the twins hinges upon whether it is symmetrical or asymmetrical. If it is symmetrical with reference to the volume of blood exchanged between the twins, all is well with both twins; but if the arterial contribution of one is greater than that of the other, or the venous

contribution of one is less than that of the other, a serious situation is sure to arise and the degree of seriousness depends on the degree of asymmetry. Which of the twins in any case is the more damaged depends upon the particular conformation of the asymmetry. The usual result of placental anastomosis is that the amount of blood which flows from the first twin to the second is not entirely the same as that which flows from the second to the first. There therefore exists in most twin placentae a dynamic asymmetry of the third circulation which is not equalized by venous anastomoses and must therefore be equalized by functional adjustments in the bodies of the twins.

Figures 50 and 51 (pp. 144, 145) represent typical placental relations in separate one-egg twins. In both there is a decided asymmetry. In Figure 50 the twin A is favored by the circulation and in Figure 51 the twin B is favored.

Although the word "favored" has been used we must understand that this term is merely relative, for it would be much better for both twins if no anastomoses of their placental circulations occurred or if the balance of exchange were equalized within the placental circulation itself. It is somewhat more immediately harmful for a twin to be robbed of part of its blood than to gain a constant access of blood from the other twin; but too much blood is in the end decidedly harmful. If we may speak of the twin which gains additional blood through the asymmetry of the third circulation as the *favored* twin and the twin which loses blood as the *injured* twin, we may discuss separately the effects of these disturbances in the two kinds of twins.

THE INFLUENCE OF ASYMMETRY OF THE THIRD CIRCULATION UPON THE DEVELOPMENT OF THE FAVORED TWIN

The primary effect of excess blood is naturally *plethora* or an overfulness of the vessels. The consequences

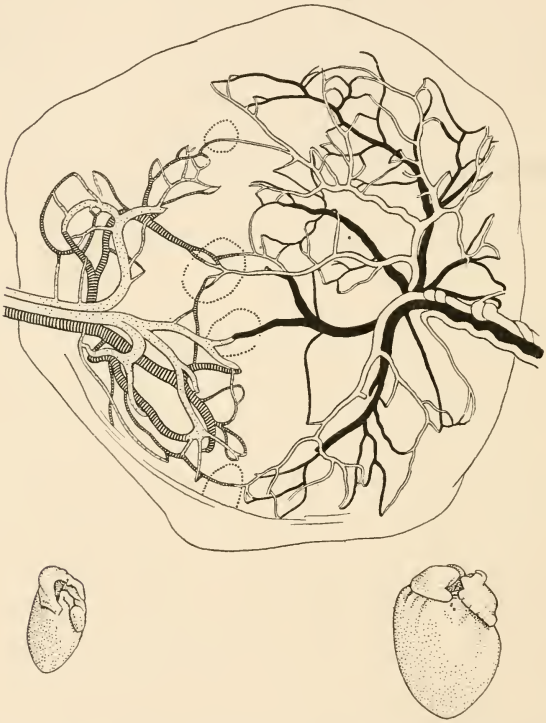


FIG. 50.—The common placenta and the hearts of a typical pair of one-egg human twin fetuses. Note the symmetrical arrangement of the umbilical cords, the superficial intertwin anastomoses of placental blood vessels within the dotted areas. The arteries are stippled on the left, unshaded on the right. The veins are cross-hatched on the left and solid black on the right. The heart of the left twin (below) is greatly reduced, that of the right is decidedly enlarged. The left-hand twin is the so-called "injured" one and the right-hand twin, the so-called "favored" one. See text for further explanation. (After Schatz.)

of plethora are: first, a general development more nearly normal than that of the injured twin; second, a heightened blood pressure in the venous system. Some secondary results are: a general oedematous and drop-

FIG. 51.—Another typical common placenta of a pair of one-egg human twins in which the vascular anastomoses, forming the so-called "third circulation" is extensive, though not greatly unbalanced. Even this degree of imbalance had very serious consequences on both twins. (After Schatz.)

sical condition of the body, the umbilical cord, and the placenta; hypertrophy, followed by atrophy, of the liver; more or less marked hypertrophy of the entire heart, which becomes not only relatively larger than that of the injured twin, but positively larger than those of single fetuses of similar size; hypertrophy of the left ventricle, observable in new-born twins: heightened

arterial blood pressure; intra-uterine opening up of the pulmonary circuit; thickening of the walls of the blood vessels, especially of the arteries; hypertrophy of kidneys and bladder; excessive urine; and excessive amniotic fluid.

All of these changes are decidedly detrimental and have, in most cases, caused the premature death or abortion not only of the injured, but of the favored twin. Only those cases in which a marked difference between the twins existed have been made the object of special study; yet one cannot help but suspect that, even in twins that are nearly equal, go to full term, and live for a considerable time after birth, some of the after-effects of minor degrees of the changes listed above may persist in one or both twins and help to account for the lower vitality and earlier death of one twin or the premature death of both twins.

Serious as are the effects of blood exchange for the favored twin, they are trifling as compared with those of the injured twin.

THE INFLUENCE OF ASYMMETRY OF THE THIRD CIRCULATION UPON THE DEVELOPMENT OF THE INJURED TWIN

The primary effect upon the injured twin is a diminution of the blood supply. In cases of only slight inequality it is likely that a somewhat lessened blood supply is a healthier condition than one somewhat increased. The after-effects are not so likely to be serious. In cases of marked inequality, however, the consequences are for the injured twin very serious indeed, because nutrition is so reduced that development is either entirely arrested or certain particular structures fail to emerge from the fetal condition. In less pronounced

cases development is at least measurably retarded. The diminished blood pressure and the lack of turgescence are often followed by definite nutritional disturbances. The kidneys, through lessened functioning, are arrested and as a consequence there is a decrease in the secretion of urine and a corresponding resorption of the amniotic fluid. This may be followed by serious mechanical consequences such as pressure of the fetus against the membranes and consequent adhesions. As a result of these physiological disturbances, intra-uterine death often occurs in one of the twins. Schatz has shown that intra-uterine death in one-egg twins occurs three times as frequently as in two-egg twins. This seems to indicate that a high percentage of the prenatal deaths of one-egg twins is to be attributed to the influences such twins exert upon each other through their intimate vascular interrelations. Probably at least half of the deaths in one-egg twins are due to causes that are independent of twinning, such as primary death of the heart or twisting of the umbilical cord. The remaining deaths are those in which we are especially interested because they are due to conditions peculiar to one-egg twins: namely, pronounced asymmetry of the placental third circulation and the immediate or secondary consequences of the latter.

HOW THE DAMAGE IS DONE

As the result of an anatomical derangement or asymmetry of the vascular system, one of the twins is robbed of the blood supply necessary for its normal nourishment and functioning. The result is a progressive weakening of the heart with an accompanying decrease in size. The pressure of the blood from the stronger opposite twin

comes to bear upon this weakened heart and, if sufficiently strong, overwhelms it and brings its rhythm to a standstill. Heart-death ensues as the direct result of its relation with the other twin. This is *secondary* heart-death. Heart-death from any other cause is *primary* heart-death and is not a consequence of twinning.

A HEART-DEAD TWIN KEPT ALIVE BY ITS PARTNER

While on the one hand a twin may injure its partner by robbing it of its blood supply and suppressing its heart rhythm, a twin which has suffered heart-death on its own account, may, on the other hand, be kept alive by its normal partner. This life-saving act is made possible by means of the third circulation. Were it not for extensive blood transfusion between the normal and the heart-dead twin the latter would die and disintegrate at once. Instead, the heart-dead twin is kept under at least partial circulation so that more or less of the body continues to develop. A completely normal individual cannot, however, be thus reared; for, in the first place, a dead heart cannot revive and therefore atrophies, and, in the second place, the circulation through the placenta from the normal twin is never sufficiently abundant nor energetic to afford nutriment to all parts of the body. Usually those organs situated on the outskirts of the zone of circulation are the first to be deprived of their needed share and are arrested in their development or become secondarily resorbed. For these reasons the prolonging of life in the dependent twin is of no value either to it or to the normal twin. In fact the vicarious heart labor consequent upon the maintenance of an

additional circulation induces heart hypertrophy in the stronger twin, and this may have serious consequences after birth when the extra burden has been removed.

Whether one twin injures the other or prolongs the life of the other we find that the most striking physiological and anatomical conditions are those that concern the heart. The heart dies and atrophies completely, it becomes weak and small in size or becomes overly strong and too large in size. We shall now consider the various heart anomalies in twins.

HEARTLESS TWINS (ACARDII)

The general term *acardia* is used to designate a condition common in twins, especially one-egg twins, characterized by atrophy of the heart. Schatz distinguishes between complete lack of heart (holoacardia) and partial lack of heart (hemiacardia). In a twin exhibiting holoacardia the circulation is carried on entirely by means of the heart of the other twin. In a twin with hemiacardia the circulation is carried on partly by the foreign heart and partly by its own heart. So long as a twin's own heart continues to function to any extent, or even if the foreign heart is only locally effective, as would be the case if the direction of the blood stream were reversed in an umbilical artery, the condition would be diagnosed not as holoacardia but as hemiacardia.

There may be as many as twenty-eight different situations depending upon which of the four types of placenta, *A*, *B*, *C*, *D* (see p. 142), are concerned and which one of the following seven types of vascular irregularity is present in a particular case.

a) slight
b) moderate } asymmetry in the third circulation
c) great

d) interruption of current in umbilical artery
e) interruption of current in umbilical vein } and simultaneous great asymmetry of the third circulation

f) primary heart-death in one twin (with pronounced asymmetry of third circulation)
g) destruction of one-half of the placenta

The effects upon the favored twin (the one that acquires an excess of blood) differ in every case from those upon the injured twin (the one that suffers a diminution of blood). In all there would appear to be fifty-six permutations and combinations of the possible variables in physiological interrelations between one-egg twins. In nearly half of the combinations either the favored, or rarely the injured, twin shows no noticeable departure from the normal; but it seems to be quite probable that many of these apparently normal individuals suffer physiologically so as to acquire certain functional heart weaknesses or disorders, and it may well be that the very common difference in vigor or vivacity between one-egg twins is the result of an intra-uterine injury of the same kind but of lesser degree than those that are clearly recognized. The most serious results are found in connection with placenta-type *D* and the vascular conditions *c*, *d*, *e* for both twins, and *f* and *g* for the injured twin. Moderately serious results appear in connection with placenta-type *A*, and conditions *b*, *c*, *d*, *e*, for the favored twin, and conditions *c*, *d*, *e*, for the injured one; and in placenta-type *B*, and conditions *b*, *c*, *d*, *e*, for the favored twin, and *c*, *d*, *e* for the injured one. So few examples of placenta-type *C* occur that the situation is less clear and may be omitted from the present discussion.

Complete acardia (holoacardia) occurs only in placenta-type *D* (in which in addition to villous transfusion, there are both arterial and venous anastromoses) in connection with vascular conditions *d* and *e* for the favored twin and *d*, *e*, *f*, and *g* for the injured twin. Hemiacardia occurs only in placenta-type *D* under exactly the same conditions as does holoacardia. Various degrees of macrocardia and microcardia occur frequently in connection with all four placenta-types and in connection with most of the vascular conditions. When one twin of a pair shows microcardia the other shows macrocardia. In Figure 50 is shown a typical placenta and the two hearts of the associated twins, that on the left being microcardiac and that on the right macrocardiac. Undoubtedly various minor degrees of microcardia and macrocardia exist in even supposedly normal one-egg twins, and these conditions may have a permanent effect on their vitality.

MORPHOLOGICAL RESULTS OF ACARDIA

Naturally we should expect that acardia would be accompanied by other more or less serious consequences; for no pronounced slowing up or deficiency of blood could occur without affecting the developing fetus. In general it may be said that the particular defects induced depend more or less directly upon the magnitude of the departure from normal and the particular type of vascular disturbance which has brought about the acardia. Schatz distinguishes the following well-defined types of acardii on the basis of their general morphology:

1. *Acardii completi*, which possess head and trunk, but may be more or less deficient in arms or legs.

2. *Acardii acormi*, which possess only the head and the merest rudiments of body and extremities (Fig. 52).

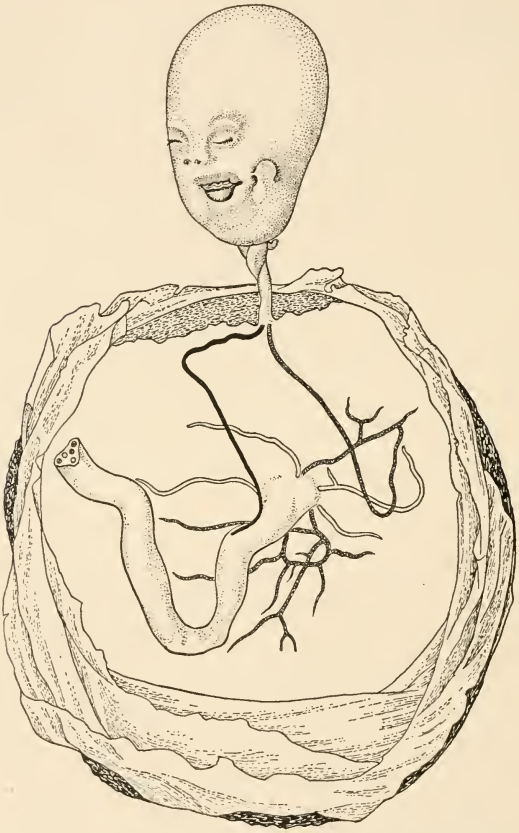


FIG. 52.—A bizarre type of heartless human one-egg twin, which had a nearly normal partner, born alive (see severed placenta of the latter turned to the left). This monster is technically an *acardius acormus* (heartless, trunkless). It is little more than a large head attached to the placenta by an umbilical cord. Such a monster is kept alive by borrowing blood from its more fortunate twin partner. (After Schatz.)

3. *Acardii acephali,* which possess a body and at least some of the extremities fairly well developed, but no head (Fig. 53).

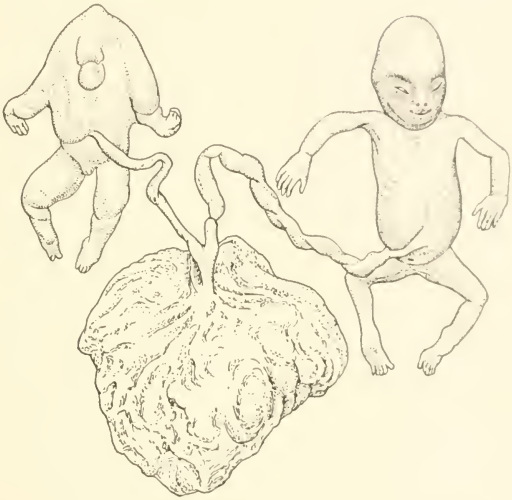


FIG. 53.—A pair of one-egg human twin fetuses attached to the common placenta. The one on the left hand is headless and has a dead heart. It is an example of the type of twin known as *acardius acephalus.* (After Schatz.)

4. *Acardii amorphi* in which the whole organism is a shapeless, rounded mass with only traces of head and appendages (Fig. 54, p. 154).

There are various more or less well-defined types of acardii amorphi, distinguished by various vascular peculiarities.

One of each of these types of acardii is shown in the accompanying figures. Some of them, especially the

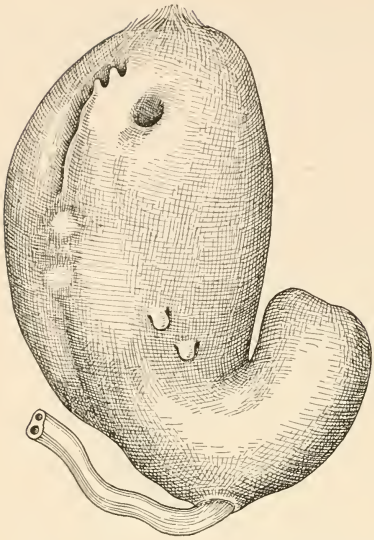


FIG. 54.—A very abnormal human twin fetus which has resulted from early heart-death and has developed badly owing to the paucity of the borrowed blood supply from the "favored" twin. Such a twin is practically unrecognizable as a human creature and is an *acardius amorphus*. (After Schatz.)

acormi, are, perhaps, the most bizarre examples of human teratoma. Schatz describes considerable numbers of such monstrous types in great detail and in each case suggests, on the basis of a study of body and placental vascular conditions, the probable cause of each. These are matters too far removed from our present interest to warrant discussion here.

Up till now we have confined our attention to conditions in entirely separate human one-egg twins. It now remains for us to make a brief survey of conditions in human double monsters or conjoined twins.

CONJOINED TWINS

ON THE INFLUENCES WHICH CONJOINED TWINS EXERT UPON EACH OTHER

Just as in separate human twins one individual may be smaller in size or defective in various ways, so in conjoined twins the component individuals may be very unequal in size and normality of structure.

In cases of marked difference between the two components the larger more nearly normal individual has come to be called the *autosite*, and the smaller more or less abnormal individual, the *parasite*. This terminology has probably certain false implications. It implies either that one of the individuals is in some sense an originally superior or primary one and that the other is a sort of secondary outgrowth of the first produced by budding, or that the parasite has come to attach itself secondarily by fusion to the body of the primary individual. Both of these alternative implications are, I believe, obviously ill founded, as will be appreciated when it becomes known that the same vascular anastomoses exist between conjoined twins as prevail for separate one-egg twins.

CONJOINED TWINS WITH SEPARATE HEARTS AND SEPARATE UMBILICAL CORDS

The majority of conjoined twins are joined only by the body wall and have most of the viscera separate. There are therefore the same opportunities for one component to injure the other through interference with its blood supply as if they were not united at all. Schatz cites a large number of instances of acardia, mediacardia, microcardia, and macrocardia in conjoined twins, and makes scarcely any distinction on the grounds of separateness or union of twins. He seems to take it for granted that the two types are simply two expressions of the same phenomenon. Without going into any great detail then, it would appear that the developmental hazards of conjoined twins are the same as those of separate twins and we may expect to find the same kinds of inhibited individuals. In cases where complete

acardia develops we may expect the injured component of a pair of conjoined twins to exhibit the most profound deterioration, equivalent to acormi, acephali, and amorphi. Thus the injured individual may be reduced to merely an extra abnormal head, an additional limb or pair of limbs, or to a shapeless mass of more or less differentiated tissues surrounded by a cyst. The opportunity for complete suppression of the injured twin is greater in the case of conjoined than in that of separate twins, because of the fact that the two are in such close contact. This relation makes it possible for the tissues of the stronger component to grow more or less completely around the weaker component and to inclose it. Thus we may have a certain amount of regulatory growth tending to obliterate the effects of incomplete twinning. Cases have not infrequently been observed in which an individual apparently quite normal has had removed from the abdominal cavity a tumor which, on examination, has turned out to be the amorphous remains of a formerly conjoined twin. Such a regulation from conjoined twinning back to a nearly normal single condition has been observed in twin starfish larvae and has already been discussed (pp. 26, 27).

CONJOINED TWINS WITH SINGLE UMBILICAL CORDS BUT SEPARATE HEARTS

There seem to be very few cases of conjoined twins that can be interpreted as having originally had only one heart. If, in advanced stages, the autosite has a heart and the parasite none we must conclude that the latter is an acardius which has secondarily lost its heart. Where there is only one umbilical cord and only one set

of umbilical vessels it is obvious that we cannot account for the inequality of the twin components as the result of asymmetry in the third circulation of the placenta. Such a condition could only be due to some asymmetry or inequality in the bodies of the components themselves. Schatz considers that one of the chief sources of initial inequality is partial *situs inversus viscerum*. He has observed in double monsters not a few cases in which a partial *situs inversus* of the heart or main blood vessels causes unfavorable circulatory relations. In complete *situs inversus* the components would doubtless be equally favorably related to their respective body parts, but a slight degree or any incomplete degree of *situs inversus* will be unfavorable. Any initial handicap, when there is competition for a single blood supply, would doubtless result in progressive gain of advantage on the part of the individual initially favored and a progressive loss of ground by the less fortunate twin. Thus might arise the conditions of autositism and parasitism.

In a previous connection (p. 67), when discussing the causes of inequality in the components of double monsters in fishes, we offered as one explanation of this inequality that there is probably as much vascular anastomosis on the vitellum of the fish as on the common placenta of human twins. Now that the reader has seen how great an interinfluence actually does exist between the components of conjoined twins and that the mechanism of this interfluence is largely one involving unequal blood transfusion between the components, he will appreciate the force of the argument against Stockard's interpretation of the cause of the differences

in size and degree of normality of the components of fish double monsters. In conclusion I would also like further to urge another explanation of the inequality of the two components that was previously offered in the case of fishes (see pp. 67, 68): that the two bilateral primordia, after physiological isolation, are somewhat independent and are not affected to the same extent by the prevailing growth-retarding agencies that have been responsible for twinning. There is just as much reason for the two bilateral components of a pair of conjoined twins to be different in their susceptibility to inhibiting agents as there is for the two sides of a single individual to be differently affected. The same causes are almost certainly concerned in the same ways in both cases.

CHAPTER XI

HEMIHYPERTROPHY—A TYPE OF MINIMAL TWINNING IN MAN

Hemihypertrophy is a relatively rare anomaly observed in human beings, which has been defined as "an overgrowth of one half or one side of the body or of a part."

Quite recently Dr. Arnold Gesell has presented an interesting and illuminating discussion of the etiology of this condition which seems to lead to the conclusion that it is, in all probability, one expression of the widespread phenomenon of bilateral twinning.

There is always a certain amount of asymmetry between the right- and left-hand sides of individuals or between the paired bilateral structures. No human being is exactly the same on the two sides, but usually the discrepancy in the two sides is not sufficient to excite comment. Occasionally, however, individuals have come to the attention of physicians and psychiatrists in which the asymmetry is very striking. In the paper of Dr. Gesell (1921) mentioned above a detailed account is given of a youth, twenty years of age, who was very strikingly larger in all the organs of the right side, head, face, arms, legs, trunk. Even such median structures as the nose and the penis were distinctly larger on the right side than on the left. Mentally this boy "must be classified as an imbecile." "The facts clearly indicate," says the author, "that hemihypertrophy should be added

to the list of developmental anomalies which bear some lawful relation to the incidence of mental deficiency."

The author tabulates forty cases of total hemihypertrophy that have been reported in the literature, and twenty-three cases of partial hemihypertrophy, a considerable proportion of which are associated with mental defectiveness. In discussing the relation of hemihypertrophy to mental defects he says:

> The frequent association of hemihypertrophy and of cranial asymmetry with mental defect and the consistent preference in both conditions for enlargement of the right side suggests some lawful grouping of causative factors. It is quite possible that the origin of certain altogether obscure cases of secondary amentia may lie in an undetectable but decisive imbalance of the fundamental process of twinning which follows fertilization.

In addition to the prevalence of mental defects in connection with hemihypertrophy there occur a number of other abnormal conditions always on the hypertrophied side. Among the commonest of these are various skin peculiarities such as surface dilation of capillaries causing red spots, large hairy moles, blue pigmentation, dark-red pigmentation, excessive hairiness. Some other peculiarities of the hypertrophied side are also of interest. The hair may be colored differently on the two sides, that of the hypertrophied side being longer and darker; the temperature may be as much as two to four degrees higher; the epiglottis shows reversed asymmetry. The fact that about 70 per cent of the cases listed involve hypertrophy of the right side suggests the possibility of symmetry reversal, but there is no data to show that there ever occurs any true *situs inversus viscerum.* The only instance of the sort

is that given above, viz., that there was noted in one case of right-handed hemihypertrophy a reversed asymmetry of the epiglottis.

An interesting discussion entitled "Twinning and Asymmetry" in Dr. Gesell's paper appears to me to be of sufficient interest to deserve quoting at some length:

TWINNING AND ASYMMETRY

It is natural that a discussion of the etiology of hemihypertrophy should finally bring us to problems of double psychical personality and twins. Further researches into the biology of twinning may bring the remarkable phenomenon of unilateral hypertrophy more completely within our comprehension; may even prove it to be on closer scrutiny more frequent and less anomalous than we had supposed. Indeed, even now, all things considered, the real marvel is not the occurrence of hypertrophy but the fact that hemihypertrophy is such an extreme rarity.

By twinning we mean the production of equivalent structures by division. This statement is taken from the biologist Bateson, who regards the power to divide as a fundamental attribute of life. The tendency to symmetry, to bilateral equivalence or mirror-imaging is so general that it must be regarded as a fundamental of biologic mechanics. Hemihypertrophy accordingly may be conceived as some profound inaccuracy in the natural process of developmental duplicity. It is not as monstrous as the double monsters, but it may have a related morphogenesis. At any rate, we can safely assume that hemihypertrophy is not an artifact really consisting in a hemi-atrophy. It is evidently a mild unilateral gigantism of an individual whose lesser somatic half is normal.

In a certain biologic sense we may regard every bilateral individual as being a pair of twins. H. H. Newman, in his fascinating work on *The Biology of Twins*, holds that monozygotic twinning —where a single egg produces two offspring—is "a phenomenon that should be considered as only a phase of the much more general phenomenon of symmetrical division. The development of the

right- and left-hand homologous organs in a bilateral organism is essentially a twinning process." This author also observes that "the whole matter of bilateral development appears to be quantitative in nature, in that the same type of process may go not so far or farther than normal." Just as there may be an inhibition of the normal culmination of the process of bilateral division (as in the median cyclopic eye), so there is frequently an excess of division resulting in two bilateral structures becoming completely segregated, as when a single individual develops two heads or two tails, while the remainder of the organism is a more or less normal individual. Newman, like Bateson, regards the phenomenon of twinning as a fundamental process which is almost universal in the field of biology.

From this point of view, hemihypertrophy may be interpreted as an atypical or incomplete form of twinning, a variant of the same process which may produce a double-headed monster or a perfectly ordinary normal individual—an ordinary individual being an organism in whom there has been a precisely balanced inhibition on the biologic process of bilateral doubling.

DISCUSSION

If we accept hemihypertrophy as a mild form of unilateral gigantism we must view the hypertrophied side as abnormal and the small side as relatively normal. From this point of view we are led to compare the condition in question with one that has been frequently noted in connection with various unilateral monstrosities in fishes which are the result of certain growth-retarding agents. It very commonly happens in such fish embryos or larvae that the organs of one side of the body develop much more rapidly than those of the other side and we get curved or even spiral forms. Frequently the eye of one side is much more defective than that of the other, and the same is true of fins and other paired structures. In view of these facts we might interpret these cases,

and perhaps also cases of human hemihypertrophy, as due to a physical inequality of the two bilateral primordia so that one side would be more affected by subnormal conditions than the other. Even in cases of double monsters there is often a pronounced difference in the two components that is probably based upon some early physiological inequality of the two bilateral primordia.

Even with this possible explanation of asymmetry in mind I find myself in essential agreement with Dr. Gesell in his interpretation of hemihypertrophy as a minimal phase of double monstrosity. We may conclude that, since twinning in general consists of a more or less complete isolation, physiological at first and later physical, of the bilateral primordia of a single embryonic axis, there may readily occur slight degrees of physiological isolation or independence of the two halves of the body. Hemihypertrophy, complete and incomplete, would then be the result of such relatively slight isolation and would therefore logically belong in the same series with duplicate twins, double monsters, and other types of twins. The unilateral hypertrophy would be thought of as the result of some deficiency confined to one side, and the associated peculiarities would be viewed as secondary consequences of the primary deficiency.

CHAPTER XII

SYMMETRY REVERSAL AND MIRROR-IMAGING IN TWINS AND DOUBLE MONSTERS

SYMMETRY REVERSAL IN HUMAN TWINS

Ever since double monsters began to attract the attention of biologists the occurrence of reversed symmetry has been a matter of marked interest. The earlier observations are confined to instances of reversal of the asymmetry of certain unpaired organs such as stomach, heart, aortic arch, vena cava. The familiar condition in which the greater curvature of the stomach is to the left, the apex of the heart to the left, aortic arch to the left, and vena cava to the right is by the older writers called *situs solitus*—the situation normal for single individuals. The unusual condition in which the greater curvature of the stomach is to the right, apex of heart to the right, aorta to the right, and vena cava to the left was at first called *situs viscerum transversus*. The older authors also use for this phenomenon the terms *situs rarior*, *solito inversus*, and *situs inversus viscerum*. Among English-speaking writers it has come to be referred to as *symmetry reversal* or *mirror-imaging*. Rare instances appeared in which a single-born individual exhibited symmetry reversal, and it has commonly been suggested that such individuals are probably surviving right-hand components of twin pairs in which the left-hand component was either formerly a parasite, afterward completely resorbed, or else had died at an early stage.

In *human conjoined twins*, especially in those known as anadidymi, in which there are two separate heads while the bodies are united in the thoracic and abdominal regions, symmetry reversal is quite common. According to Spaeth (1860) there are all degrees of *situs inversus viscerum* ranging from cases of complete reversal to cases in which there is only a trace of it. Many cases of slight symmetry reversal are overlooked, but it is these marginal cases, which, according to Schatz (1887), involve the greatest danger for the twins possessing them. They are nearly always associated with some irregularity in the circulatory system and such irregularities are almost certain to have serious consequences when the blood supplies of the two individuals come to be united by placental anastomoses. Not only are there numerous instances of *situs inversus* in conjoined twins but, according to Spaeth and Schatz, small deviations from the normal situs are often found in separate one-egg twins. Such deviations do not so much concern the larger more obvious organs, such as stomach, heart, aorta, but have to do especially with less conspicuous regions of the circulatory system.

Schatz in studying separate human one-egg twins, especially the acardii dealt with in chapter x, noted many instances of incomplete *situs inversus viscerum*. In one case the only reversal present had to do with the chief vessels of the heart; the aorta was on the right, the vena cava on the left. In another case the aorta was to the left of the vertebral column yet to the right of the vena cava. Another case was noted in which there was practically bilateral symmetry of certain normally asymmetrical structures. Even these lesser

departures from the normal *situs solitus* are to be interpreted as evidences of incomplete symmetry reversal. Complete *situs inversus* in separate one-egg twins is rare; but one conspicuous instance has come to my attention. This is the case of Kuchenmeister (1883) who describes a pair of nearly full-term twins one of which had complete *situs solitus*, the other complete *situs inversus*.

FIG. 55.—A striking example of a human double monster (anadidymus) which had perfect *situs inversus viscerum*. (After a photograph published by Morrill.)

In this place it is not my purpose to present any large amount of data on *situs inversus viscerum* in human twins. Morrill (1919) has recently given us a concise survey of the literature on this subject. He himself has made a detailed study of a double-headed monster that came to his attention and has figured the monster in its entirety (Fig. 55) as well as its visceral conditions (Fig. 56). This is obviously a case of complete *situs inversus viscerum* and is of very great interest. Morrill calls attention to the existence of *situs inversus* in the cases of several rather famous diplopagi. Eichwald states "that the thoracopagous monsters examined by him showed, in almost every case, some transportation of the viscera in one of the bodies, though to a varying extent." The pygopagous Carolina twins, Millie-

Christina (colored), are declared to have had *situs inversus* of at least the heart. One of the famous Siamese Twins is stated to have had a partial reversal of viscera.

A few significant conclusions may be stated in reviewing the matter of symmetry reversal in human twins:

a) The condition is obviously associated with a form of twinning in which the two individuals are derived from a primordium that would normally produce the right- and left-hand halves of a bilateral individual.

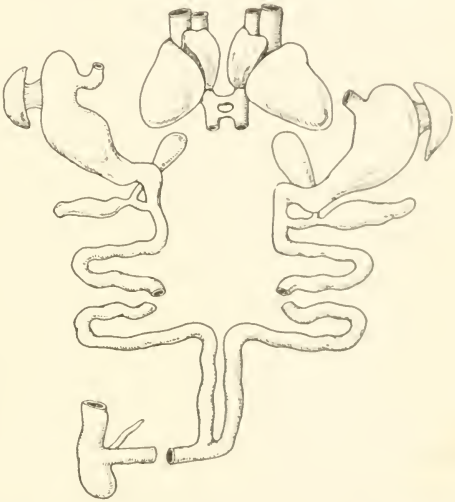


FIG. 56.—The viscera of the double monster shown in Fig. 55, laid out in somewhat schematic fashion to show the complete *situs inversus viscerum*. (From Morrill.)

b) The left-hand individual always retains the normal situs and the right-hand individual very commonly shows some degree of symmetry reversal.

c) It is very rare indeed that separate human twins show any signs of *situs inversus viscerum*, but they do not uncommonly show mirror-imaging in certain minor external characters such as finger-prints (see *The Biology of Twins*, pp. 159–60).

d) If we include minor degrees of symmetry reversal we find it to be a much more frequently occurring phenomenon than we had formerly supposed. Complete *situs solitus* in right-hand components of conjoined twins is, in fact, relatively rare.

e) The existence of mirror-image symmetry in the components of double monsters argues against the theory that they have arisen through the fusion of separate embryonic axes and for the theory of origin through dichotomy or fission of the bilateral halves of a single embryonic axis.

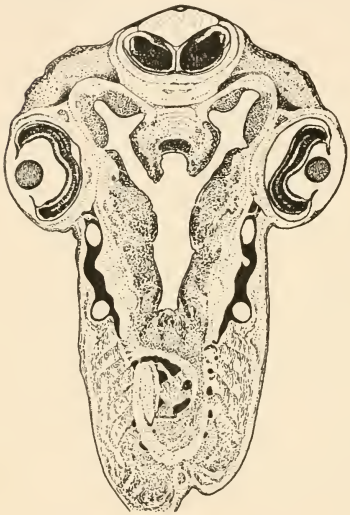


FIG. 57.—A horizontal section through the head region of a partially double trout embryo. The embryo is double as far back as the anterior end of the mid-brain. Note the perfect mirror-imaging of the twinned structures. (After Gemmill.)

SYMMETRY REVERSAL IN FISH TWINS

Until very recently no attention seems to have been paid to symmetry reversal in fish twins. Gemmill (1912), although he figures in great detail numerous conditions that are essentially symmetry reversals, never calls attention to the condition as such. One cannot help but note the beautifully perfect mirror-imaging displayed in his horizontal sections through the heads of partially double-headed

monsters (Fig. 57). Each side is a practically exact mirror-image of the other, the inner sides in each partly divided head being less fully developed than the outer. We note another equally beautiful instance of mirror-imaging in the skulls of double-headed monsters. In Figure 58 is shown such a skull in which all outer bones in both components are perfect and a number of inner bones imperfectly duplicated or single.

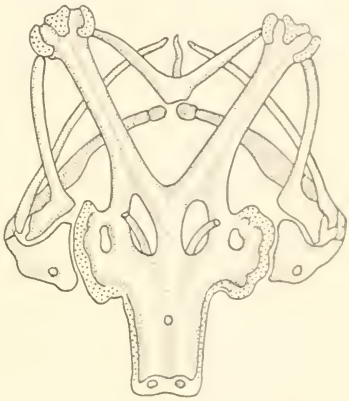
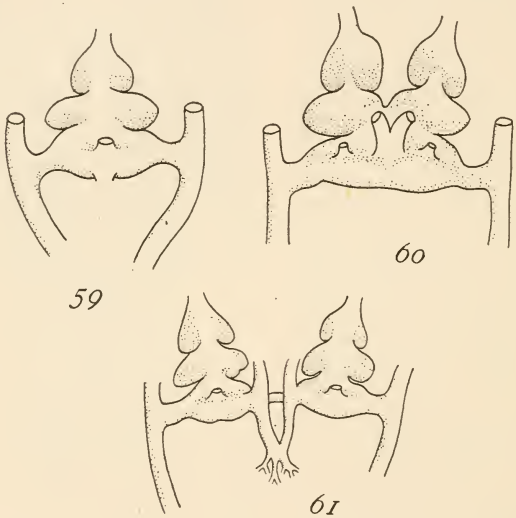


FIG. 58.—The skull and jaw of a partially double-headed young trout, showing perfect mirror-imaging of twinned structures. (After Gemmill.)

Certain beautiful cases of duplicity of visceral parts are also figured, such as heart and the urogenital system. Especially interesting are the conditions in some of these hearts in which various degrees of duplicity are shown. Figure 59 (p. 170) shows the heart of a normal fish embryo before it has assumed any marked degree of asymmetry. Figure 60 (p. 170) shows a case of partial duplicity, especially complete at the ventricular end. Figure 61 (p. 170) is an example of almost complete duplicity of the heart, only the inner vessels being united, especially the inner vena cava. The two halves are perfect mirror-images. One step farther and the two hearts with their main vessels would be entirely separated the one set from the other. Were such a further separation to occur we would

expect a continuance of the mirror-imaging. Gemmill does not give us any further data on this point, but two recent studies on inverse symmetry in double-monster trout have supplied this deficiency.

Morrill (1919) has worked on the internal anatomy of some of Stockard's collection of double trout. He



FIGS. 59–61.—Three diagrams showing symmetry reversal in the hearts and principal vessels of trout double monsters. Fig. 59 shows the normal single heart. Fig. 60 shows an incompletely twinned heart. Fig. 61 shows completely divided twinned hearts, the right component of which shows *situs inversus viscerum*. (After Gemmill.)

found several cases of complete symmetry reversal and also several cases that were incomplete or doubtful. Never was there any symmetry reversal in the individuals in which body and tail were both separate, but only in cases in which heads were entirely separate and the viscera separate at least back to the pelvic region.

In the cases of typical *situs inversus* the left-hand component shows the normal situs and the right-hand component the reversed situs of all asymmetrical structures. Morrill states, however, that mirror-imaging, even in the types of duplicity described as most favorable for its appearance, is by no means the rule, but that the majority of specimens show the normal situs in both components. On the basis of this finding he concludes that reversal of symmetry is rare in fishes.

Somewhat more recently Swett (1921) has studied the subject in another collection of somewhat more advanced trout embryos. He also finds that *situs inversus* is confined to monsters showing a certain limited range of duplicity. Swett says:

> It will be seen that those whose vertebral columns are fused anterior to the dorsal fin and those that are nearly separate show at best only doubtful *mirror-imaging*. It is further noted, as was also found by Morrill, that not all the animals falling within the limits of doubling apparently most favorable for transposition of the viscera show this phenomenon. Thus there are indications that the reversal of asymmetry is not a necessary consequence of any condition of doubling, but may or may not occur, depending on some other factor capable of operating within these limits.

Certain additional facts appear in Swett's paper. He finds that mirror-imaging of the digestive tract exists only when the point of union of the twin tracts occurs at some point between the pylorus and the point of exit of the intestine from the abdominal cavity. Another point of considerable interest, in view of the interpretation given by Stockard of the nature and relationship of autosite and parasite in double-monster fish, is that in Swett's material there is a case of partial *situs inversus* in the parasite (Fig. 62, p. 172). This finding goes far

toward corroborating my theory that even two unequal components arise through the separation of the bilateral primordia of a single embryonic axis, and that one half must have been more susceptible to retarding agents than the other. A very clear case of typical and complete *situs inversus* is shown in Figure 63 (p. 173). Several other good cases are figured by Swett. It may also be said that, even in a much smaller collection of double monsters,

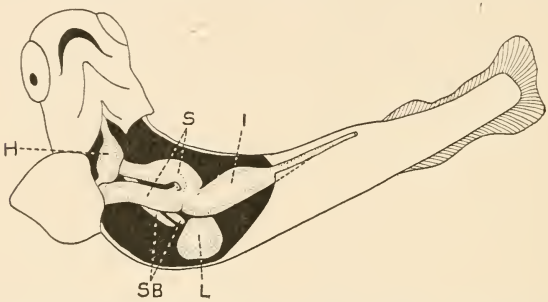


FIG. 62.—Ventral view of trout double monster of the autosite-parasite type, showing partial *situs inversus viscerum*. The lobe at the left of the figure just below the normal head is the reduced parasite component representing the inferior or right-hand twin. *H*, heart; *L*, liver; *SB*, swim bladder; *S*, stomach; *I*, intestine. (From Swett.)

Swett found more cases of *situs inversus* than did Morrill in a larger collection. The difference, I think, is largely one of interpretation. Many slight degrees of reversal are likely to be overlooked. In all probability the cases described by Morrill as uncertain or irregular should be diagnosed as examples of incomplete *situs inversus*.

In attempting to interpret the data on *situs inversus* in fishes two particular conditions need to be emphasized:

1. There is no reason to expect to find *situs inversus* except in double individuals derived from the antimeric halves of a single embryonic axis. In the case of

embryos derived from two embryonic shields, each of which has its own right- and left-hand sides, we see no reason in the world why there should be a reversal of the viscera. Each is from the beginning a separate

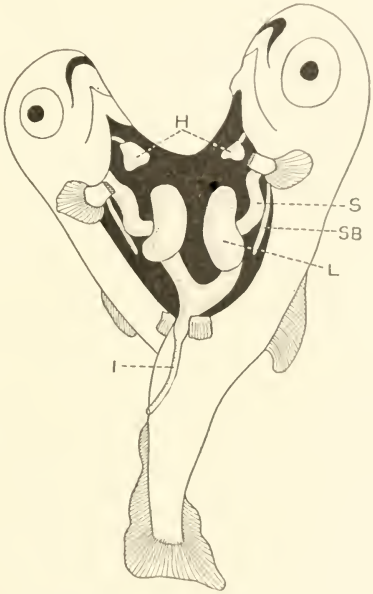


FIG. 63.—A ventrolateral view of a trout double monster (anadidymus) showing *situs inversus viscerum*. The component on the reader's right is the true left-hand component and has the typical left hand asymmetry of the species. The component on the reader's left (the right-hand component) has the reversed asymmetry of the viscera Letters have the same significance as in Fig. 62. (From Swett.)

individual except that it happens to be on the same yolk sac as its twin partner. It would also be impossible to say which of such twins is the right-hand and which the left-hand component. Therefore right-handed or reversed asymmetry would be quite inappropriate in

either one of them. If we were ever to find *situs inversus* in twins separate except for the common yolk sac, it is certain that the explanation of such an occurrence would offer a problem of extreme difficulty. Normal situs in both of these twins, which I have classed as separate one-egg twins, is, then, only to be expected. The same statement may be made for those double monsters that are united only by lateral or ventral parts. Such individuals may be interpreted as resulting from secondary or mechanical fusions due to the close proximity of two embryonic shields. Unless double monsters have a considerable single region which is like that of a normal fish, I would say that such forms do not come from the antimeric halves of a single axis and therefore would not be expected to show *situs inversus*.

2. In the well-defined cases of twins with rather extensive axial regions in common, such as the types in which *situs inversus* occurs, our problem is not so much to account for *situs inversus* (for this is the expected condition in such bilaterally related twins) as to account for the not infrequent occurrence of left-hand asymmetry (*situs solitus*) in the right-hand component. This seems to be the real symmetry reversal, for the right-hand component is expected to be a mirror-image of the left, and should show right-hand asymmetry (*situs inversus*) of asymmetrical structures. The process is probably one of regulation. The right-hand component, which in some cases retains the symmetry of the half-primordium from which it came, may become sufficiently independent to develop its own asymmetry just as though it were a single individual in no way influenced by its twin component. Abundant instances were

noted by the writer in the case of armadillo quadruplets in which both twins showed the same unilateral asymmetry in their scute peculiarities. It was about equally common, however, for them to show mirror-image symmetry. Here we have a somewhat parallel case and the explanation is the same: that there is in some cases a greater degree of isolation between the twin components than in others, so that sometimes the two individuals act as though entirely independent, and in others as though they still retained some residuum of the earlier interrelationship characteristic of antimeric halves of a single individual, a relation that expresses itself most obviously in mirror-imaging.

In concluding this discussion of mirror-imaging in fish monsters I would like to reiterate what appears to me a very fundamental principle: that *mirror-imaging is normal for twins derived through separation of the antimeric halves of a single embryonic axis.* Such individuals rarely, possibly never, become entirely separate. There is no reason to expect *situs inversus* in separate twins that have originated from two complete embryonic axes on a single blastoderm.

EXPERIMENTAL PRODUCTION OF *SITUS INVERSUS VISCERUM* IN TRITON

A recent very significant paper by Spemann and Falkenburg (1919) has thrown much light on the problem of reversed symmetry. The object of this investigation was to discover what would be the symmetry relations in twins artificially produced by severing the blastula or gastrula stage down the sagittal plane. This operation effectually isolates the right- and left-hand primordia

of a blastoderm. The amphibian embryo has a high capacity for regeneration and hence a very large proportion of the half-embryos regenerated so as to produce each a whole individual. As a rule, however, the regenerated half did not grow so rapidly nor differentiate so completely as the older half. The result is that the right side of the embryo derived from the left-hand piece and the left side of the embryo derived from the right-hand piece were usually imperfect in various ways. We may speak of the regenerated side of both twins as the inner side. Now it happens that on the inner side the appendages are always smaller, the eyes sometimes smaller, and the body musculature much less developed. As a consequence of the lack of body musculature the bodies of the twin embryos are often concave on the inner side and may be even spirally coiled. In this way it often happens that structures on the two outer sides are similar and those on the two inner sides equally similar. This is a sort of mirror-image asymmetry that might be readily explained as the result of imperfect regeneration of the side that had been lost; but it is not this rather obvious sort of mirror-imaging that attracted the interest of these authors. The really striking discovery was that out of the thirty right-hand pieces that survived exactly half grew into larvae that showed *situs inversus viscerum;* the other half showed normal left-hand asymmetry. This discovery is rendered even more striking by the fact that out of twenty-five surviving left-hand pieces none showed definite reversed or right-hand symmetry. One left-hand piece there was in which a slight irregularity of the heart was noted, that might have been interpreted as a case of minimal reversed symmetry.

It would be very remarkable if the left-hand pieces developed into larvae with right-hand situs, for the species shows typically only left-hand asymmetry, as in man and the fishes. There is, however, some cause to emphasize the frequency of *situs inversus* in the right-hand pieces. Even those right-hand twins that were not classed as definite cases of *situs inversus* showed what we may legitimately, I think, consider as cases of partial *situs inversus*, since they were scarcely at all asymmetrical (i.e., lacked the normal left-hand asymmetry) or had only a slight degree of left-hand asymmetry. On the whole, then, we may say that, as a rule, the right-hand pieces show more or less *situs inversus* or the asymmetry which we would expect to find in an individual derived from the right-hand primordium of a blastoderm that had already established its axis of symmetry.

Spemann, in an elaborate discussion of the causes of *situs inversus* in these experimental twins, seems inclined to refer the normal specific (left-hand) asymmetry back to certain asymmetric relations of a molecular sort in the egg. He presents as an analogy to the foregoing experiments certain facts brought out by Przibram in connection with crystals. Certain asymmetrical crystals, after one side has been injured by cutting, rebuild themselves so as to be the mirror-image duplicates of the typical crystal. It is suggested by Przibram that this reversal of asymmetry is due to reversal of the microstructure of the crystals, possibly involving actual molecular changes. One finds such an analogy extremely attractive and almost unescapable whenever an attempt is made at an ultimate analysis

of organic asymmetry. As yet, however, the theory far outstrips the facts.

Using this idea as a working hypothesis we might readily conceive of a condition in which, because of the fundamental molecular asymmetry characteristic of a group of animals, one side of the body (the left in vertebrates) is the superior side in a physiological sense. This side normally grows more rapidly so as to produce curvatures or unilateral hypertrophies of certain median organs; or else certain other organs, ordinarily paired, grow only on the left side. The right side is the inferior side and, in the presence of the left or superior side, is kept in some sort of subordination. If some agency lowers the dominance of the superior side the inferior side might become independent and might develop its own symmetry relations without hindrance from the superior side.

Let us examine the facts of *situs inversus* in Triton twins in the light of this theory. These isolated right halves will, during the period of regeneration, have a superior side of their own, the outer or right side, and this side will more or less completely dominate the regenerating left side. Thus the molecular asymmetry is doubtless established in a reversed direction. In some right-hand pieces, however, there may be sufficient of the left-hand material present to set the molecular symmetry more or less completely to the left, thus producing the typical situs of the species. This theory of a superior and an inferior side in vertebrates is in accord with many different facts. We know, for example, that man is typically right handed because the motor centers of the right-hand musculature lie chiefly on the left side of the brain. The abnormal condition of hemihypertro-

phy in man strikes the right or inferior side in about three-quarters of the cases. Amphioxus is quite asymmetrical, with many structures on the left or superior side that are lacking on the right. We shall now examine a parallel situation among the echinoderms where unilateral asymmetry is very marked indeed, but still capable of experimental reversal.

REVERSED SYMMETRY IN ECHINODERM LARVAE

In sharp contrast with the vertebrates, in which unilateral asymmetry is at best only slight, stand the echinoderms in which certain structures of the left side grow so much more rapidly than those of the right that they almost crowd out the corresponding right-hand structures altogether. The early echinoderm larva is, at least morphologically, bilaterally symmetrical, and it is only in relatively advanced larval stages that the left side begins to show its superiority over the right. During the development of the coelomic pouches a left-hand hydrocoele appears, which has no counterpart on the right side. This hydrocoele is the primordium of the madreporic pores and pore-canals and of the radial water-vascular system. In sea urchins the presence of the hydrocoele also stimulates the development of the so-called amniotic invagination on the left side, which has no counterpart on the right.

SPORADIC INSTANCES OF BILATERALITY IN ECHINODERM LARVAE AND THEIR SIGNIFICANCE

One of the favorite anomalies of the invertebrate embryologist is the occasional larva in which the hydrocoele and its accompaniments appear on the right side as well

as on the left. This appearance of bilateral symmetry in the occasional larva has been interpreted by McBride and others as a reversion to an ancestral state, and is believed strongly to support the theory that the echinoderms, at present a radially symmetrical group, have been derived from a bilaterally symmetrical ancestor through the gradual suppression of the coelomic structures of the right side.

Recently the writer (1921, *a*), while engaged upon the experimental production of twins in the starfish *Patiria miniata* discovered a culture of advanced bipennariae in which the majority of individuals had both right and left madreporic pores and pore-canals. A typical specimen of this culture is shown in Figure 64. These specimens were nearly always strictly bilaterally symmetrical, but a few showed a slightly less perfect condition of the madreporic canal or pore on the right side than on the left. It should be emphasized that these bilaterally symmetrical larvae were found in a crowded culture in which development had taken place under somewhat subnormal conditions. It previously had been found that when large numbers of eggs were allowed to develop in a single vessel there always occurred a considerable number of various types of twins. When, therefore, along with a considerable number of twin larvae, there appeared a great many of these bilaterally symmetrical larvae, it was natural to look upon this condition as a kind of twinning. The idea then was that any doubling of normally single structures might be interpreted as twinning, and, therefore, animals with paired madreporic pores and pore-canals, instead of the single pores and canals, were essentially twins. I now

look upon this situation in a somewhat different light. While still holding that the appearance of bilaterally symmetrical larvae is a sort of twinning phenomenon, I believe that it is really a phase of symmetry reversal. In the typical larva, with but one hydrocoele complex

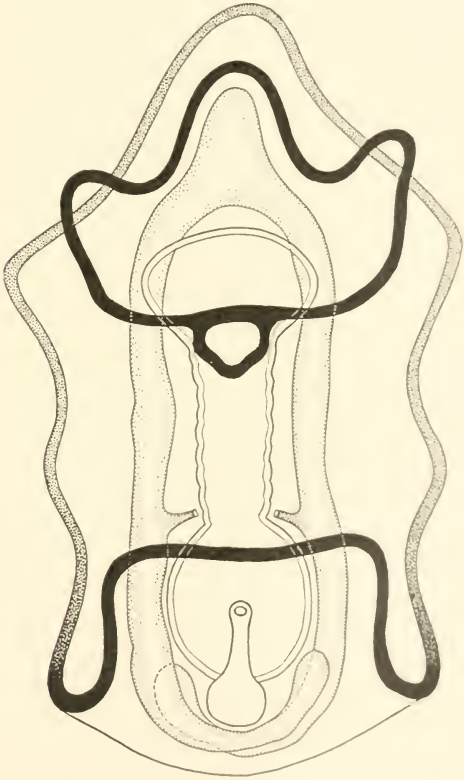


FIG. 64.—Ventral view of an advanced bipennaria larva, one of many observed by me, of the starfish *Patiria miniata*. The water pores, pore-canals, and hydrocoele vesicles are symmetrically developed on both sides. (Original.)

on the left-hand side, it seems quite evident that there is a pronounced superiority of the left side, for the left side develops many important structures that normally do not appear on the right side at all, but are certainly potentially represented on that side.

We are driven to the conclusion that the appearance and rapid development of the hydrocoele structures on the left side inhibits in some way the development of equivalent structures on the right. It is well known that an actively growing region may inhibit the development of other actively growing regions, as when a terminal bud in a plant inhibits for some distance back of it the growth of lateral buds. If, however, such a terminal bud is cut off, or if in some way its rate of growth is retarded, the lateral buds are free to go ahead. In some such way as this I would interpret the physiology of the bilaterally symmetrical condition in the starfish larvae above described. Normally the left side, especially in the region where the hydrocoele develops, has a more rapid rate of development than has a similar region on the right side. The left side may be compared, therefore, to a terminal bud which inhibits the development of equivalent growths on the right side. If, however, the developmental rate of the embryo is checked at some critical period when the asymmetry of the two sides is being established, the discrepancy between the two sides fails to appear and they start their hydrocoele differentiation simultaneously as twin hydrocoeles, neither of which is dominant over the other and therefore neither one is inhibited.

McBride (1918) succeeded in producing a considerable number of bilaterally symmetrical larvae of *Echinus*

miliaris by subjecting the eggs and early larvae to hypertonic sea water. In spite of the fact that the bilateral condition is experimentally produced under abnormal conditions, he adheres to his view that the development of a right-hand hydrocoele in addition to the normal left-hand one, is "an indication that the common bilateral ancestor of the Echinodermata had, corresponding to the hydrocoele, a paired organ equally developed on both sides of the body, and that, whilst the organ on the left side became further developed until it grew to be the water-vascular system and its appendages, the organ on the right side dwindled and disappeared."

It seems more logical to me to conceive of the ancestral echinoderm as a simple bilateral organism which through some mutational change in the germinal protoplasm acquired an asymmetry which enabled only the left-hand hydrocoele to develop. This originated the first step in radial symmetry which culminated in the condition now present. When both sides develop hydrocoeles we have a condition equivalent to twinning, which can hardly be thought of as an ancestral reminiscence. Moreover, we now know that instances of reversed symmetry are almost as common as cases of bilateral symmetry in echinoderm larvae, and it would be impossible to consider a right-handed individual as ancestral to a left-handed one.

CASES OF SYMMETRY REVERSAL IN ECHINODERMS

A significant paper by Oshima (1921) has recently appeared in which that writer gives an account of the discovery in certain laboratory cultures of *Echinus mili-*

aris of "a number of abnormal plutei which had the hydrocoele developed on the right side instead of in its normal position on the left side." He calls attention to the fact that several other authors had previously noted instances of reversed symmetry in echinoderms. Runnström (1912) had found this condition in two individuals of *Strongylocentrotus lividus;* Müller (1850) had long ago described auriculariae with hydrocoele on the right side only; while Mortensen had observed two plutii of *Ophionotus hexactis* showing similar conditions.

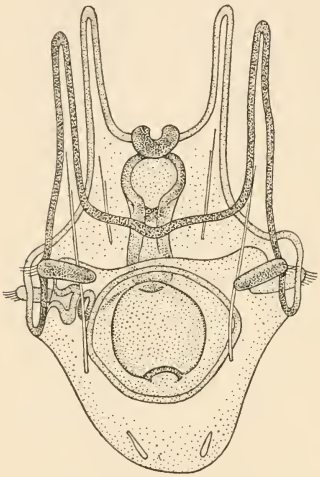


Fig. 65.—Ventral view of a pluteus larva of the sea urchin, *Echinus miliaris*, showing complete reversal of asymmetry in the hydrocoele, which appears on the right side only instead of on the left, the situs typical for the species. (From Oshima.)

The purpose of Oshima's experiments was to repeat McBride's method of producing larvae with double hydrocoeles. Using the latter's procedure he obtained about 10 per cent of larvae exhibiting *situs inversus* (Fig. 65) and about 2 per cent with double hydrocoeles. Curiously enough, however, the control cultures, which had not been treated with hypertonic sea water showed the same percentage of anomalous forms. It must not be forgotten, however, that the cultures were reared in artificially mixed sea water and that the food was scanty. These conditions were sufficient to account for the

peculiar condition. Oshima's own explanation of the condition is as follows: "The growth of the normally developing hydrocoele might have been arrested from some cause and the right anterior coelom, to compensate this defective development, produced a new hydrocoele on the right side." To me the condition is simply an exaggeration of that in which bilaterally symmetrical hydrocoeles are produced. In some way, as the result of abnormal growth conditions, the development of the left-hand hydrocoele is inhibited, while the right-hand hydrocoele becomes physiologically isolated and begins to grow before the left has sufficiently recovered to resume development. The right-hand hydrocoele now inhibits the left because it has a higher rate of development, and is therefore the superior side. Thus we have before us a case of complete symmetry reversal experimentally induced.

A GENERAL THEORY OF THE PHYSIOLOGY OF SYMMETRY REVERSAL

Any theory of symmetry reversal must be founded upon a correct understanding of the physiological status that exists in a perfectly bilaterally symmetrical organism. I look upon such an organism as the resultant of an intimate interplay and co-operation of two systems of primordia which are in focus upon a single median plane, a plane which is equivalent to a sagittal plane of the adult organism. The two antimeric halves are in a sense twin individuals with a common apical plane. So close is the co-operation or integration of the two halves, however, that one half influences the opposite half in such a way that equivalent structures appear in the two

halves as mirror-images of each other, just as the two halves of a symmetrical crystal are mirror-images. If, in our hypothetical perfectly bilaterally symmetrical organism, the two halves were to be physically or physiologically isolated, we would expect exactly equivalent twins, for each half-primordium would regenerate in such a way as to reproduce the exact bilateral symmetry present in the original individual of which they are parts.

As a matter of fact, however, a certain amount of unilateral asymmetry appears to be characteristic of most organisms. In some organisms such asymmetry is very pronounced, as in echinoderms and in gastropod molluscs, while in other organisms it is much less pronounced, as in vertebrates and in many arthropods. Whether the unilateral asymmetry affects many organs or a few, whether the extent of the asymmetry be great or little, the basis of the asymmetry seems to be one involving a physiological superiority of one side or the other. In last analysis the difference between the two sides may be reduced to terms of rate of fundamental vital activity, probably measurable in terms of rate of oxidation. The result is that one side develops rather more rapidly than the other, especially in connection with certain structures that arise near the median axis or mirror plane. Thus, in vertebrates, the left side of the stomach, of the heart, and of other median structures grows more rapidly than the right; and certain other structures, such as swim-bladder in fishes and left aorta in mammals, appear only on the left and not on the right. Similarly, in echinoderms the left side of the larva develops more rapidly than the right, and certain

structures, the hydrocoele and its derivatives, start to grow first on the left. In both of these groups we believe that the earlier onset of rapid growth in certain left-hand primordia inhibits more or less completely the growth of equivalent structures on the right. The phenomenon of growth inhibition in these and other allied cases is probably bioelectric in character. If so, the region of rapid growth at any level of the primary axis is positive to regions of less active growth and there is a one-way bioelectric current, which furnishes the medium of control of one part over another. R. S. Lillie has demonstrated similar relations in connection with metals in solutions of electrolytes. One positive or anodic region seems to have an inhibiting effect over a given distance so that other similarly charged regions cannot arise near the original anodic region. Whatever be the ultimate physiology of the inhibition exercised over one growing region by another more actively growing region, the actual fact of inhibition is beyond question.

If now we in some way break down the co-operation of the two half-primordia destined to form the bilateral halves of a single individual, the two halves become more or less completely independent, and twinning results. If the twins are separated down the whole axis, or if they are separated as far back as the posterior end of the body cavity, the two severed halves will each undergo complete regulation, each forming a whole organism with the symmetries and asymmetries characteristic of the species. Thus completely divided human twins, armadillo twins, and fish twins rarely if ever show *situs inversus viscerum*, but always possess the left-hand asymmetry characteristic of the species.

If, however, as in the fishes, the process of bilateral fission happens to halt at a certain definite level, where the alimentary tract is divided pretty well back of the stomach, but remains single in a considerable part of the intestine, it seems to be a matter of touch and go whether the right-hand component of the conjoined twins will regulate in such a way as to take on the normal left-hand asymmetry of the species (*situs solitus*) or will continue to behave, with regard to some of its structures, as though it were half of a single bilaterally symmetrical organism. The condition seems to me to be much like that exhibited by bilaterally symmetrical echinoderm larvae in which, the dominance of the left-hand hydrocoele having been reduced, the right-hand half assumes equivalence and both develop equally as bilaterally symmetrical structures. In the cases in which *situs inversus viscerum* is found in conjoined twins of the fishes, we may interpret the effect as due to a lowering of dominance of the left-hand side over the right only to the point where they are equally dominant, each being to a slight extent influential over the other. In other words a certain degree of the old bilateral integration of the two half-primordia remains to express itself in the mirror-image relations of the viscera. When the co-ordination is completely broken down the right-hand individual, as well as the left-hand one, regulates the normal asymmetry of the species. There appears then to be a very delicate equilibrium at some period, in connection with bilateral primordia destined to produce twins, between a condition of complete isolation and a condition of partial integration between the two halves.

In separate one-egg human twins mirror-imaging seems to persist mostly in certain integumentary structures such as friction-ridge patterns on index fingers, occasional reversals in direction of whirl in the crown of the head hair. Yet there are not a few instances in which one twin is right handed, the other left handed. As in the case of fishes, however, the normal condition in completely separate twins is a complete regulation in both individuals of the specific asymmetry of all structures. In the armadillos, especially in the case of twins derived from a secondary blastoderm of one side, the incidence of mirror-imaging is more frequent, there being nearly as many instances in which some asymmetrical integumentary peculiarity is found on opposite sides of a pair of twins as on the same side. Here again the equilibrium at the time of separation of the twin primordium must be extremely delicate and some very minor factor may decide whether the two individuals shall both show unilateral asymmetry of the same side or whether one shall be the mirror-image of the other. In these cases we cannot speak of symmetry reversal because we do not know with regard to any sporadic asymmetry of the scute pattern what is the specific condition or the *situs solius*. All we can say is that both individuals are mirror-images of each other. In the next chapters are discussed further cases of mirror-imaging that must be taken into account in reaching any final judgment as to the causes and significance of symmetry reversals.

CHAPTER XIII

DOUBLE TAILS IN VERTEBRATES

Among bilateral animals of all sorts it is very common to find, instead of the single tails or limbs characteristic of the species, double tails or limbs. Such double structures are unsually known as *duplicities*, but are really cases of local twinning, as I shall attempt to show.

Double tails have been very frequently described in connection with experiments on regeneration of lost tails. Not uncommonly one finds in lizards that the regenerated end of the tail has grown out more or less completely doubled. Similar results have been reported in connection with regenerated tails of various Amphibia.

Some years ago when the writer (1915*b*) was engaged in an extensive series of hybridization experiments upon the bony fishes it was noted that in some crosses, a considerable percentage of the hybrid larvae had double tails. This was especially true in the cross *Tautogolabrus adspersus* ♀ × *Stenotomus chrysops* ♂ (Cunner ♀ × butterfish ♂). This cross furnishes a very extensive assortment of monstrosities among which there were both double-headed and double-tailed individuals. The double-tailed ones were, however, very numerous. Occasional double-tailed individuals were found in the various other crosses, especially in the cross between the eggs of the butterfish and the sperm of *Fundulus heteroclitus*, where one perfectly symmetrical double tail was found. In all of these experiments the double-tailed condition was found associated with various

other abnormalities such as cyclopia, humpback, abnormal heart. It seems only reasonable, then, to infer that the same types of causes are responsible for the excessive growth seen in double tail as are responsible for the defects above named. In all cases it is very plain that the rate of development from an early period has been decidedly retarded as compared with that seen in the corresponding pure-bred embryos.

DOUBLE-TAILED GOLDFISH

One species of fish is characteristically double tailed even in nature—the goldfish (*Cyprinus auratus*). The production of the double-tailed conditions is, like that of other morphological oddities in these fishes, under the control of the breeders, who are experts in these matters. Two particular kinds of double tails are common: (*a*) those in which each half of the double tail is essentially a complete tail and the two tails lie side by side, only united dorsally at the point of their union with the body; (*b*) those in which the two tails are more or less completely fused by their dorsal margins in such a way that the double fin is a three-lobed affair. All stages of complete and incomplete doubling occur in any lot of fishes derived from one batch of eggs. Sometimes the doubling involves the vertebral column and sometimes only the fin rays or vertebral arches.

The condition of double tail does not seem to be definitely heritable in goldfishes. Normal parents produce many offspring with double tails and double-tailed individuals produce many normals. There is inherited, it seems, merely a high degree of susceptibility to the conditions responsible for doubling. What these are we shall now inquire.

THE CAUSES OF DOUBLE TAILS

Tail doubling is in my opinion a phenomenon very similar to head doubling, but is probably characteristic of a later developmental period.

Thanks to the recent experiments of Dr. Hyman (1921) we now know that the fish embryo, during a relatively early germ-ring stage, forms the rudiments of a tail-bud, the equivalent of the *Knopf* of Kopsch. This posterior region of the primary axis has from an early time a very high relative susceptibility to inhibiting agents, such as anaesthetics, lack of oxygen, cold. Even in presomite stages of the embryo there is present a double gradient, similar to that in annelid worms, with points of high activity and susceptibility at the anterior and at the posterior ends and with gradients of susceptibility running both backward and forward. After the anterior parts of the axis are fully established and have undergone considerable differentiation, the posterior end of the axis remains an actively growing region. It is evident that after a period of retardation this actively growing tail-bud region undergoes bilateral fission in order to form double tails. Abundant evidence is at hand indicating that the twinning process of the tail is due primarily to a slowing-down of the developmental rate so that the two bilateral halves lose their coordination and proceed independently, each regulating for itself a bilateral symmetry more or less complete. Mirror-imaging is under these conditions quite the expected thing, and the expectation is always realized. Thus we see that twinning of the tail is extremely like twinning of the head and body, and doubtless depends on the same factors operating at a different time.

CHAPTER XIV

TWINNING (DUPLICITY) IN LIMBS

As long ago as 1894 Bateson, in his classic work *Materials for the Study of Variation*, devoted two chapters to "Supernumerary Appendages in Secondary Symmetry." A representative series of instances of supernumerary appendages such as antennae, palpi, and legs is described, involving many groups of insects, crustacea, and vertebrates. These extra appendages may be either entirely separate outgrowths near the normal appendage, or, as is the case in the majority of instances, they may occur as outgrowths from an appendage—such as extra legs growing from normal legs. In both types of cases the symmetry of the supernumerary appendage appears to bear a definite relation to that of the neighboring appendage or to the one upon which it grows. It is quite common to find that the supernumerary appendage growing from another appendage is itself a twin appendage in which the two parts are mirror-images of each other. A very pretty case of this relation is seen in Figure 66,

FIG. 66.—An example of limb duplicity in an insect, *Pterostichus mühlfeldii*. The component to the right is the normal tarsus. The extra tarsus on the left is duplex and shows mirror-image symmetry. (After Bateson.)

an anomalous leg of a beetle. On the basis of a large number of such instances Bateson is able to set down certain rules of symmetry:

When extra appendages, arising from a normal appendage, are thoroughly relaxed and extended, the following rules will be found to hold good with certain exceptions to be hereafter specified:

I. *The long axis of the normal appendage and the two extra appendages are in one plane:* of the two extra appendages one is therefore nearer to the axis of the normal appendage and the other remoter from it.

II. *The nearer of the two appendages is in structure and position formed as the image of the normal appendage in a plane mirror placed between the normal appendage and the nearer one, at right angles to the plane of the three axes; and the remoter appendage is the image of the nearer in a plane mirror similarly placed between the two extra appendages.*

The symmetry between the nearer double member and the normal appendage may be called primary symmetry and that between the two twinned members, secondary symmetry. The terms major and minor symmetry are sometimes used quite synonomously with these.

In a book of the present scope it would neither be desirable nor feasible to enter into an exhaustive survey of the elaborate literature on duplicities and symmetries in limbs. Hence we shall confine ourselves to a few selected phases of the subject that appear to be especially helpful in our attempt to analyze the phenomena of organic symmetry and symmetry reversal.

EXPERIMENTAL PRODUCTION OF DOUBLE LIMBS IN AMBLYSTOMA LARVAE

Harrison (1920) has succeeded in producing a large number of double and triple limbs in the larvae of the common salamander Amblystoma by transplanting

limb-buds at an early stage to another part of the body. He used a small circular punch by means of which he was able to cut out a little circle of the body wall of the embryo from the region known to contain the primordia of the fore limbs. These little circles of embryonic tissue were then placed in various positions (hind part before, upside down, and diagonally) in wound beds of the correct size that had been cut out of the body wall at other places than those normal for the development of limbs. Sometimes a right-hand limb-bud was put on the left side, and vice versa, in the various positions stated above. These small transplanted limb primordia produce limbs in their new positions, but they show varied results according to the side on which they are transplanted and the position in which the pieces were placed. In general it may be said that a graft tends to produce a limb with the same symmetry relations that it would have had if left where it originally was, but that there is more or less complete symmetry reversal in some cases. The normal limb grows backward, but if a limb-bud is transplanted hind-part-before the limb will grow forward. The palmar surface of the limb tends to form on the side turned toward the body of the animal and the ulnar border tends to be dorsal. Harrison says:

> The above circumstances determine the asymmetry of the limb as follows: when the dorso-ventral axis is not inverted, the original prospective asymmetry persists; when the axis is inverted, the asymmetry is reversed. In more general terms: the asymmetry of the limb is determined by two factors, the polarization of the anterio-posterior axis of the limb-bud and the orientation of the limb-bud with respect to the dorso-ventral polarization of its organic environment.

This reversal of symmetry reminds one of the symmetry reversal seen in twins and double monsters. It will be remembered that in those cases there was a delicate equilibrium between the internal factors and the external factors. Sometimes the internal factors, the inherent tendency for a twinned right-hand component to assume the characteristic left-handed asymmetry of the species, prevails; sometimes the external factor, the close proximity of the left-hand component and a tendency to integrate with it, causes the right-hand component to act as though it were merely a right-hand mirror-image duplicate of the left-hand component, and we have symmetry reversal.

Our interest in Harrison's work is, for the moment, limited to the phenomenon of limb-doubling. Double and triple limbs arise frequently from the transplanted limb-buds. These are of all grades of completeness and occur under a great variety of different experimental conditions. They occur most commonly, however, when the buds are transplanted farthest away from their normal positions. In most cases of double limbs Harrison is able to distinguish an original (primary) and a secondary limb which he conceives of as arising as a lateral outgrowth from the primary.

This symmetry relation is evidently an extremely fundamental phenomenon and in many respects reminds one of bilateral symmetry and of symmetry reversal in conjoined twins. While in conjoined twins there are many exceptions to mirror-imaging, due to a regulation on the part of the partially separated structures back to the specific asymmetry, there are in the case of these double limbs very few exceptions to the mirror-image rule. These

exceptions are, however, significant, since they represent the equivalent of the failures to show mirror-image symmetry in twins and are doubtless due to similar causes. Bateson notes two clear-cut cases of doubled limbs in beetles in which the double appendage branching from the single normal appendage has no symmetry relation to the single appendage, but the twin parts are quite distinctly symmetrical between themselves. This is really a breach of the first rule of Bateson, that "the long axis of the normal appendage and the two extra appendages are in the same plane." Various other authors have cited occasional exceptional cases, but it must not be forgotten that the symmetry rules of Bateson hold with respect to a very high percentage of all cases.

The analysis of symmetry relations in reduplicated limbs of Amblystoma, as given us by Harrison, has been rendered vastly more difficult than need be owing to the complexity of experimental procedures. Had the author been interested primarily in the study of double limbs and their symmetry relations he could have eliminated a great many complicating factors, such as the changing of grafts from right to left side or the turning of grafts upside down. Under the circumstances, it appears to me remarkable that so definite a result was obtained, and we need hardly despair about the possibility of clearing up this problem if efforts should be directed definitely to that end. Certain facts may be gleaned from a survey of Harrison's work that aid us in our present analysis: (*a*) The broad axis of the limb is at right angles to the sagittal plane of the body. (*b*) The ulnar border of the limb is dorsal and the radial border ventral, i.e., the little-finger side of the hand is

dorsal and the thumb side is ventral. (*c*) The palm surface of the limb is posterior and the back of the hand is anterior.

With these points of orientation in mind, we are now in a position to compare and contrast the twinning situation as it presents itself in conjoined twins and in double limbs. In conjoined twins we found that the organic symmetry relations were influenced only by internal factors so that each bilateral half was situated in a position exactly equivalent to that of the other. In a limb-bud, however, the environmental relations of the body to which the limb-bud belongs clearly exercise an influence upon the symmetry of the limb. If a limb-bud were to be transplanted to the median dorsal region, so that its dorsal half fell on the right and its ventral half on the left of the primary axis of the embryo, I suspect there would grow a perfectly bilaterally symmetrical limb with no difference between radial and ulnar sides and no difference between little finger and thumb. Growing as it does, however, the limb-bud cannot be bilaterally symmetrical because the ulnar border of the limb (little-finger side) is dorsal, and therefore has a higher rate of metabolism than has the radial or thumb side. Physiologically the ulnar side is the superior side. There is therefore an asymmetry quite similar to that in the whole body of such animals as the echinoderms.

If the superiority of the ulnar side should be broken down in any way we might expect to get an equivalence of superior and inferior sides something like that seen in conjoined twins with mirror-image symmetry, or like the starfish larvae with paired hydrocoele structures.

This is, I believe, just what happens when a limb-bud undergoes simple twinning. In such a twinned limb the mirror plane, as in other cases of symmetry reversal, is adjacent to the inferior or radial side of each com-

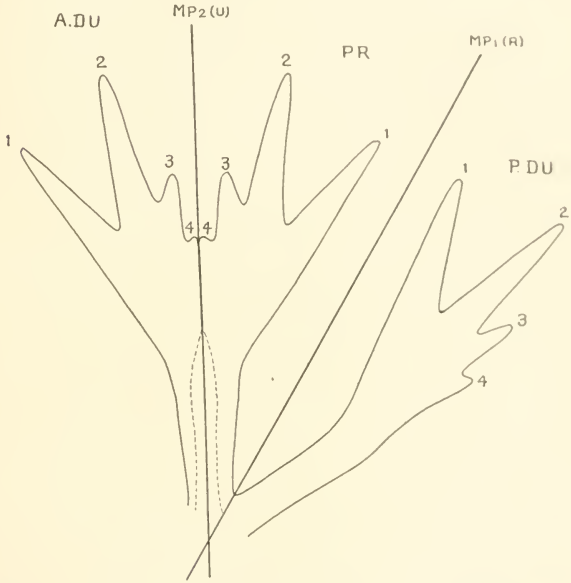


FIG. 67.—Diagram showing mode of limb reduplication (twinning) in Amblystoma. *PR*, primary limb; *P.DU*, posterior reduplicating member; *A.DU*, anterior reduplicating member; $MP_1(R)$, primary (radial) mirror plane; $MP_2(U)$, secondary (ulnar) mirror plane; 1–4, first to fourth digits, respectively. (After Harrison.)

ponent of the double-monster limb and the superior side is away from the mirror plane in each instance. Such a double limb as this is represented in Figure 67, and should be compared first with a mirror-imaged double monster, such as that of the trout (Fig. 63) and then with the completely double hand shown on page 202 (Fig. 71).

The situation is greatly complicated, however, when one of the components of the twinned limb undergoes a secondary reduplication. The mirror plane is then on the ulnar or superior side of the twinned limbs. It could not be otherwise without doing away with the mirror-imaging between the first pair of twin components, for they could not all three have the mirror plane on the radial or inferior side. This secondary reduplication undoubtedly greatly complicates matters and renders the analysis of symmetry reversal extremely difficult. It is easy to state the rule according to which mirror-imaging works out, just as both Bateson and Harrison have done, but it is not nearly so easy to account on physiological grounds for what is so readily formulated.

It is clear that the complicating factor is the second step in limb-doubling. If we could find a material in which limb-doubling was simpler we could perhaps obtain a less complicated situation that would admit of more ready analysis. It is fortunate that we have just such a series of instances of simple limb-doubling in human hands and feet.

DOUBLE HANDS AND FEET IN MAN

Bateson (1894) has described and figured a number of significant instances of hand and foot anomaly which seem to me to help us to bring the phenomenon of limb-doubling into line with bilateral twinning. These will be listed in a logical series:

1. The minimal case of mirror-imaging or break-down of dorso-ventral asymmetry of the left hand is one in which the thumb is essentially the same as the little

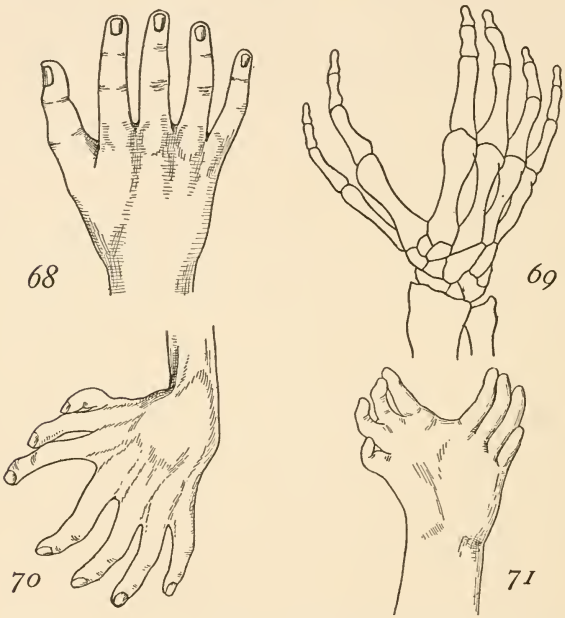
finger, though a little larger. It has three joints like the little finger and takes its origin from the palm at the same level as the latter (Fig. 68, p. 202).

2. We next have an instance in which the physiological isolation of the two sides of the hand was more pronounced, so that partial twinning has occurred. This is a case of a woman who had six digits on each hand and foot. In each hand the thumb has three joints like a little finger. There are, however, some irregularities that complicate the case.

3. Another case is cited of a hand with six digits arranged in two groups that were somewhat opposable to each other (Fig. 69). Digits II, III, IV, V stand in normal position as the ulnar set, while the radial group consists of two normal fingers, each with three joints, and neither one thumblike. Here we have partial twinning and partial mirror-imaging, but the radial side is still somewhat inferior. This case seems to indicate that in a hand of normal type the thumb stands over against the rest of the fingers as a reduced or inferior half of the appendage. The physiological isolation of the weaker from the stronger side allows the thumb side to become more nearly equal to the little-finger side.

4. A still more nearly double hand is one described from a specimen in the Harvard Medical School Museum. The forearm consists of two ulna bones instead of a radius and an ulna. In other words, the superior of these twin bones is repeated on both sides. The hand of this double arm consists of seven digits in two groups, an ulnar group of four very normal digits and a radial or thumb group of three nearly normal fingers (Fig. 70).

5. A case of complete double hand with perfect mirror-image symmetry is described by J. J. Murray. This hand, shown in Figure 71, appears to have been completely bilaterally symmetrical and without thumbs,



FIGS. 68–71.—Various degrees of duplicity (twinning) of the human hand. Fig. 68, a hand with a thumb like a little finger. Fig. 69, a hand with thumb represented by two fingers. Fig. 70, a hand with thumb represented by three fingers. Fig. 71, hand completely twinned with the thumb represented by four fingers which are mirror images of the fingers in the radial component. (After Bateson.)

a whole set of four fingers having arisen in the place of a thumb. The two sets of four were physiologically equivalent in use and value. I look upon this case as the logical culmination of the series. If our theory is correct, that the plane of symmetry in the vertebrate

hand falls between the thumb and the index finger and that the thumb is the reduced equivalent, on the radial side of the limb, of the four fingers on the ulnar side of the limb, we could not have a double hand with more than eight digits. No instances of more than eight digits have come to our attention, and whenever there are eight digits they occur in two mirror-image sets.

CASES OF SYMMETRY REVERSAL

In the case of double-headed twins, one component, always the one on the right (the individual belonging to the inferior side) may become sufficiently independent to resume the specific asymmetry (*situs solitus*). Somewhat equivalent changes may occur in the case of double hands and feet. An interesting double right hand with eight fingers is described and figured by Giraldes, in which the outer digit of the ulnar or superior side is decidedly thumblike while that on the radial side is distinctly a little finger. This is like the complete reversal of asymmetry seen in the sea urchin plutei described by Oshima (1921). Another similar case is noted by Athol Johnson, but this time in a foot instead of a hand. It is a case of a left foot with nine toes, one only partially subdivided. The four digits on the little-toe side (homologous with the little-finger side of the hand) had four normal digits, none of which is a great toe. The five toes of the great-toe side had the largest digit, much like a great toe, on the outside. The third and fourth digits of this side are only partially divided. This looks like another case of symmetry reversal on the part of the component of the weaker side back to the specific asymmetry of the single limb.

It seems highly probable that all vertebrates undergo processes of bilateral doubling such as these described. Essentially the same conditions have been shown to apply in many cases among the arthropods. These and the vertebrates are the only groups that have well-defined limbs. I am aware that our attempt to effect an analysis of the problem of symmetry reversal is not an unqualified success, but I am convinced that this point of view has elements of value and will help in the ultimate solution of a very perplexing problem.

THE CAUSES OF LIMB-DOUBLING

If limb-doubling is equivalent to that of bilateral twinning it should have the same general causes. It is our present theory that limb-doubling is caused by a lowering of the developmental rate at the time when the limb-bud is beginning its process of outgrowth or before the terminal elements have become differentiated. Now what evidences have we at present that limb-doubling is associated with developmental retardation? There are two independent lines of evidence. The first is that double limbs most frequently occur in connection with regeneration processes in which we know that, prior to regrowth, there takes place a nearly complete dedifferentiation of tissues and a loss of axiate organization. It commonly happens in regeneration experiments that two heads grow out in place of one and similarly the embryonic rudiment of a regenerating limb is likely to lose its unity and become double. Regeneration processes are usually less rapid in their early stages than are normal developmental processes, and require a reaxiation process before they can go ahead. The second

piece of evidence is of an entirely different sort and arises out of data furnished by Detwiler (1920). Using the methods of Harrison, previously referred to, this author transplanted limb-buds of Amblystoma from their normal position to various levels more posterior. Many of these transplanted limb-buds underwent doubling and showed mirror-image symmetry. According to Detwiler, "there occurred a gradual increase in the number of reduplications as the limbs became transplanted farther and farther away from the normal situation." If we take this statement in connection with another series of facts, it becomes significant; for the farther away from the normal position the graft is placed the slower it is to develop. Thus we have the greatest frequency in limb-doubling when the developmental rate of the limb-bud is most retarded; and the causal basis of limb-doubling is seen to be in all probability the same as in other more typical forms of twinning.

CHAPTER XV

TWINNING AS A MODE OF REPRODUCTION

In view of the confusion that prevails at present regarding twinning processes I believe that the following attempt to classify the various modes of reproduction, including the several types of twinning, will help to clarify the situation.

One school of writers finds the broadest distinction among modes of reproduction to lie in whether they are *sexual* or *asexual*. All types of reproduction in which there is no union of gametes are lumped together into the category of agametic or asexual reproduction as over against gametic or sexual reproduction.

In his textbook, *Principles of Zoölogy*, Shull (1920) expresses himself as follows:

> Asexual or non-sexual reproduction includes all those methods of reproduction which require but a single parent for the reproduction of offspring and do not involve germ cells. Sexual reproduction as a rule involves two parents and the production of two kinds of germ cells, the eggs and sperms. It is usually brought about by union of a sperm cell with an egg, or less commonly by the development of the egg without union with the sperm.

He then proceeds to classify modes of reproduction essentially as follows:

I. Asexual reproduction

a) Budding $\begin{cases}\text{external}\\ \text{internal}\end{cases}$

b) Fission $\begin{cases}\text{longitudinal}\\ \text{transverse}\end{cases}$

c) Sporulation

II. Sexual reproduction

a) In Protozoa { isogamy / heterogamy

b) In Metazoa { the typical fertilization process / parthenogenesis / paedogenesis / hermaphroditism

Such a classification, while in most respects logical, is incomplete, and fails to take account of mitosis, twinning, and polyembryony. At the risk of being considered venturesome, I wish to present for consideration another classification of modes of reproduction that seems to me, in some respects at least, more workable than those previously proposed.

MODES OF REPRODUCTION

I. Axiate reproduction

A. Unicellular { *a*) Mitosis in both Protozoa and Metazoa / *b*) Amitosis in both Protozoa and Metazoa

B. Multicellular

1. Transverse fission { *a*) In embryos / *b*) In adults
2. Lateral budding
3. Twinning { *a*) Fission of blastoderm before axis of symmetry is laid down / *b*) Production of two (or more) axes through plural gastrulation / *c*) Bilateral fission of the right- and left-hand primordia of a single embryonic axis

II. Non-axiate reproduction

A. Agametic { *a*) Gemmulation / *b*) Stotoblast formation / *c*) Sporulation

B. Gametic { *a*) Parthenogenetic
  i. Paedogenetic
  ii. Adult
b) Syngamous
  i. Isogamous
  ii. Heterogamous

I. AXIATE REPRODUCTION

The characteristic feature of axiate reproduction is that it involves a separation, either complete or incomplete, of a single axiate individual into two or more individuals in such a precise fashion that the line of separation between the two products of division bears a definite relation to at least one of the structural or functional axes of the original individual. There are, according to Child, three types of axiate organization among organisms, which are, in the order of their evolutionary origin and their ontogenetic development: (*a*) the radial axis or that involving a gradient of metabolic activity from the center to the surface; (*b*) the axis of polarity, involving a gradient of metabolic activity running from anterior to posterior end or from animal to vegetal pole of single cells; (*c*) the axis of symmetry, involving a double gradient running laterally from either median dorsal or median ventral sides, and giving rise to bilateral symmetry.

There is a type of axiate reproduction associated with each of these three types of axiate organization.

A. UNICELLULAR AXIATE REPRODUCTION

Doubtless the most primitive as well as the most universal type of axiate reproduction is that exhibited in the reproduction of cells. All cells, no matter how little differentiated they may be in other respects, have at least a center-surface organization and it is this axis that determines the sequence of events seen in both mitotic and amitotic cell division whether in Protozoa or in Metazoa. In this type of reproduction the essential feature is that division or doubling of cell structures

begins with those occupying the dynamic center of the cell and proceeds from the center to the periphery, culminating in a constriction of the cortex and the cell membrane. In amitotic division the nucleolus, originally single, divides into two nucleoli, which migrate apart to opposite sides of the nucleolus, thus becoming physiologically isolated; the nucleus then divides and the two halves migrate apart; and finally the cytoplasm becomes at first physiologically, and later physically, separated or isolated into two independent masses. In mitosis the centrosome and astrophere seem to constitute the dynamic center of the cell and to take the initiative in cell-reproduction. Two centers arise instead of one and these migrate apart, each organizing about itself a radial system. The chromosomes, at first occupying a neutral position between the two radial systems, divide longitudinally so that half of each chromosome migrates to each daughter-system; and finally the cytoplasm is partitioned off into two independent or semi-independent systems and cell-reproduction has been accomplished.

Cell-division in both Protozoa and Metazoa may be either equational or differential. When, as in the fission of the Mastigophora and in the majority of cases of early cleavage, the line of separation (cleavage furrow) is meridianal, i.e., parallel to the axis of polarity of the cell, the cell products are, as a rule, equivalent, each being a mirror-image of the other. This may justly be termed *cellular twinning*, for it strikingly resembles the most characteristic types of twinning seen in the higher organisms and differs only in that the process is concerned with but one cell instead of many. When, however, the line of separation is at right angles

to the axis of polarity, as it is in the majority of free Infusoria and in the first cleavage of Ascaris, Unio, and similar forms, the process is much more like the transverse fission seen in flatworms and in metameric groups. It differs from twinning in that the daughter-cells are not equivalent and in a lack of mirror-imaging between them.

B. MULTICELLULAR AXIATE REPRODUCTION

1. *Transverse fission.*—This type of reproduction involves the cutting off of a new individual by means of a fission plane at right angles to the primary axis. As a rule a new organism is cut off from the basal region of an individual which is elongated in and growing in a posterior or basal direction. Transverse fission is mainly characteristic of free-living as opposed to sessile organisms, in which a posterior zoöid, if cut off, is free to break away and lead an independent life. Examples of transverse fission are seen in the formation of new zoöids in planarians, in strobilation in the cestodes, and in metameric segmentation in the embryos and larvae, of annelids, arthropods, and vertebrates. In the more primitive types of transverse fission the newly formed individual breaks away from the old and regenerates a new anterior end or head, becoming ultimately a complete individual. In the case of strobilation the individuals produced by fission, though they sooner or later become independent, are never able to regenerate the head, and hence remain incomplete. In metameric segmentation the new individuals are incompletely cut off and remain attached, as subordinate zoöids, to the original head, and the whole series becomes secondarily integrated into a single organism. Later on some of

these metameres specialize so as to become sexually mature and gametic reproduction takes place. In a very real sense, therefore, the process of segmentation may be said to represent an asexual phase in the life-cycle of a metameric animal.

2. *Lateral budding* is the characteristic asexual method of reproduction of sessile or sedentary organisms in which one end of the primary axis is permanently or semi-permanently attached to the substratum. We find budding of this kind in Porifera and Coelenterata, the two lowest metazoan phyla in which there is no axis of bilateral symmetry. The characteristic feature of this type of reproduction is that at some level of the primary axis a new growing-point arises that has acquired an independence from the dominance of the apical end. This new apical point grows out essentially at right angles to the main axis, although subsequent flexures may alter the angle between the two axes. The new growing-point becomes the apical end of a new zoöid and this proceeds to organize its own basal parts. In the coelenterates the formation of a series of asexual zoöids into a colony is essentially like what happens in the case of strobilation or in that of metameric segmentation. In both cases the younger zoöids remain sexless, but in later stages at least some of the zoöids become sexually mature and produce gametes.

3. *Twinning.*—Twinning is a precocious form of axiate reproduction involving a physiological isolation of bilaterally symmetrical halves of the blastoderm and the consequent physical separation of a single individual into two equivalent parts. Gross fragmentation of a blastoderm without reference to axiate relations, even if it gives

rise to several independent embryos, is not twinning. Twinning is essentially a dichotomy, a division of one primordium into two. Repeated dichotomies may give rise to numerous offspring from a single blastoderm as in the South American armadillo, *Dasypus hybridus;* or there may be only two dichotomies as in *Dasypus novemcinctus*, or there may be one complete dichotomy, followed by a partial dichotomy of one twin, as in certain cases of so-called triple monstrosities in fish. Twinning is essentially a physiological isolation of two equivalent growing-points due to a partial loss of integration of the bilateral halves of the blastoderm. The completeness or incompleteness of the process depends upon the degree to which the dominance of the original apical end has been suppressed. Twinning differs primarily from both transverse fission and lateral budding in that it is an affair of the axis of symmetry, instead of the axis of polarity.

II. NON-AXIATE REPRODUCTION

This type of reproduction has apparently no direct reference to the axiate relations of the parent organism. Small masses of tissues or single cells are isolated in various ways from the parent tissues—usually internal tissues—and are capable under the proper environmental conditions of reproducing new organisms like the parent. It is probably true that the tissues that give rise to gemmules, statoblasts, spores, or gametes, are so related to the axes of the parents that physiological isolation is favored. In that sense we might consider all reproduction as, in last analysis, axiate, but for our purposes the axiate relation is so vague, if present at all, that we are justified in ignoring its existence.

1. *Agametic non-axiate reproduction.* Little need be said about the modes of reproduction included in this category. They have sometimes been considered as cases of internal budding, because groups of cells are cut off instead of single cells as in gametic reproduction. There are probably various gradations between agametic and gametic reproduction. In the case of polyembryony in the parasitic hymenoptera it seems to be true that at first small groups of cells become isolated from the embryo or polygerm and, after two or three generations of somewhat gross fragmentation of the polygerm, single cells become isolated and develop into the definitive sexual embryos. Thus polyembryony seems to involve both agametic and gametic non-axiate reproduction.

2. *Gametic reproduction.*—The characteristic feature of this type of reproduction is that single, more or less specialized, cells (gametes) are formed which, on isolation, are capable of reproducing a whole organism. The period at which gametes may be isolated varies greatly in different groups of animals. When the isolation takes place in an embryonic, larval, or juvenile stage, we speak of the condition as *paedogenesis.* One of the most interesting life-cycles, involving very early paedogenesis, is that of the liver fluke. In this species of parasitic flatworm the very young ciliated larva, the miracidium, bores its way into the soft tissues of a snail and there grows into a bag-like vesicle called the sporocyst. The sporocyst produces within its cavity a number of cells which behave exactly like parthenogenetic gametes; for each cell undergoes maturation and goes through a regular process of cleavage resulting in a larva of a different sort, called a redia. These redias in turn

reproduce much in the same way for some generations until finally a generation of cercarias is produced. These find their way out of the snail and into the liver of the sheep, where each slowly transforms itself into an adult fluke.

There are several species of paedogenetic insects in which reproduction takes place in the larval condition. Paedogenesis is recognized in some of the vertebrates also, as in the classic Axolotl and probably in the perennibranchiate urodeles. In fact paedogenesis grades over almost imperceptibly into full adult reproduction. In very early paedogenesis the reproduction is of necessity parthenogenetic, but in later paedogenesis regular syngamous reproduction takes place. Just as there is a gradation between agametic and gametic reproduction, so we have a graded series of stages ranging from extremely early paedogenesis to late adult syngamous reproduction. Parthenogenesis is evidently a phase of gametic reproduction and its incidence is capricious; we find it here and there throughout the animal kingdom from Protozoa to Chordata. It is not to be thought of as a reversion to a primitive mode of reproduction but rather as an evidence of racial senescence.

THE COMMON FEATURE OF ALL REPRODUCTIVE MODES

The one connecting link between the various modes of reproduction is the principle of *physiological isolation*. So long as the individual is completely integrated in all of its parts it will not reproduce, but if for any reason the integrative forces that hold together the various parts into a single organism weaken, either a general or

a local breaking away or emancipation of parts occurs, and the isolated parts become the beginnings of new organisms. If the integrative forces are moderately lessened or inhibited, only certain less completely integrated or outlying parts tend to gain physiological independence. Functioning independently tends further to isolate and, in the end, the part becomes not only physiologically, but physically independent. Then follows the process of reconstituting the specific form, and this is able to take place whether the isolated part is a single germ cell, a small mass of internal cells, a lateral bud, the isolated halves of a bilateral primordium, or the posterior half of an axiate animal.

TWINNING AND ALTERNATION OF GENERATIONS

Among the lower animals it is very common to find that the life-cycle is much more complex than in the higher animals. In the colonial hydroids, for example, an egg develops a young hydranth which by lateral budding produces a whole series of similar asexual hydranths. Late in the season certain budded individuals, medusa buds, develop into free-swimming sexual individuals, medusae, and these produce eggs and sperm, which in turn unite to give us the fertilized egg again. We thus seem to have an alternation between an asexual mode of reproduction and a sexual, which has been called *alternation of generations*. Without seriously opposing the value of thus marking off what is essentially a continuous ontogeny into separate generations, I wish to enter a protest against what appears to me to be a misuse of the facts of twinning, especially that in the armadillos. Twinning is, in my opinion, a cenogenetic

or a senescent condition and in no sense a reversion to an ancestral condition. Therefore it is a mistake to consider that twinning in any sense represents the now generally lost asexual phase in the life-cycle of the vertebrates.

Gemmill (1912) is doubtless the originator of this mistake as may be judged from the following passage:

> The view has often been suggested that the blastoderm may be looked upon as a stock, able to give rise vegetatively, so to speak, to more than one embryo. The natural comparisons have been drawn between this faculty and the alternation of generations which occurs normally in some groups of lower animals and in plants. It has been sought to recognize alternation of generations in the development of all animals. More probably, however, in animals, twinning, double and triple monstrosity, polyembryony, and alternation of generations, provide instances in which a common "potentiality" has become realized, and beyond that are not necessarily connected by any nexus of a direct or phylogenetic character.

This is at least a non-committal statement of this point of view, but it has evidently led to a considerably less cautious statement by Stockard, who says:

> The suggestion has frequently been made that the blastoderm may be looked upon as a stock able to give rise asexually to more than one embryo. Since the natural process of budding to form four or more embryos in the armadillo is recognized, and accessory individuals may be produced experimentally from other vertebrate eggs, it becomes evident that even man and the highest animals may actually at times exhibit an alternation of the sexual and asexual processes of reproduction.

If any real significance is to be attributed to finding among vertebrates a similitude of the so-called alternation of generations of the lower forms, one need hardly go so far afield to discover it. Instead of tracing it back to a typically sessile and radial group such as the Coelenterates, we can find it among the Rhabdocoels,

a free-living group with bilateral symmetry. Such types as the planarians and Microstomum illustrate alternation of generations very beautifully. For a long time, while relatively young, they reproduce by transverse fission and after a series of fissions reproduce sexually. In the annelids first, and then in the vertebrates, we have the equivalent of the asexual period (characterized by transverse fission) in the process of metameric segmentation. The agamic period is pushed farther and farther back in the life-cycle until in the highest vertebrates it is completed during the first few days of development. The introduction of cases of embryonic twinning in order to supply the missing link in an ideally universal alternation of generations is, therefore, to say the least, gratuitous. Moreover, if this view were carried out to its logical conclusion, we would be led to admit that wherever we find cases of doubling of normally single individuals or parts of individuals we have reminiscences of a lost asexual generation. Such a theory would undoubtedly seem absurd if applied to cases of doubling of tails or doubling of limbs, which begin at relatively late stages of ontogeny. It seems just as absurd to designate the partial fission of the bilateral primordia of a single embryonic axis as a reminiscence of a lost asexual generation. Yet such processes are due essentially to the same factors as is twinning in the armadillo. At the risk of seeming pedantic, therefore, I would once more suggest to biologists the need of caution in assigning phylogenetic values to cenogenetic processes, such as one-egg twinning in armadillos and in man, or to experimentally induced twinning in fishes.

POLYEMBRYONY AND TWINNING

It is an unfortunate circumstance that one-egg twinning in the armadillos was described first as a case of polyembryony. The fact that all embryos derived from a single egg were of the same sex strongly reminded the discoverers of twinning in the armadillos of the condition described by Sylvestri and others for the parasitic hymenoptera, where sometimes hundreds of embryos were produced from a single egg and all embryos from any one egg were of the same sex. Since the hymenopteran condition had with considerable appropriateness been called polyembryony, it was not unnatural that several of us who first studied the armadillo situation should adopt for it too the term polyembryony. At that time we did not fully understand the exact mode of origin of the multiple embryos in either parasitic hymenopteran or in the armadillo. Now that both conditions are adequately understood, it is clear that the armadillo case is a clear-cut instance of dichotomous twinning, a form of axiate reproduction; while true polyembryony, as seen in such forms as Paracopidomopsis (Patterson, 1921) involves nothing at all equivalent to the processes of twinning, but follows a mode of reproduction essentially non-axiate, resembling much more closely the paedogenetic type of reproduction of the liver fluke than it does twinning in the armadillos. In Paracopidomopsis there are evidences of several embryonic generations that remind one of the several larval generations in the liver fluke, Fasciola. The cleavage period results in a so-called "morula stage," which loses its axiate relations and becomes a mass of generalized cells called a "polygerm." This soon becomes subdivided

into fifteen or twenty primary cell masses. Since there is evidently some integration among the cells of these primary cell masses, they may be considered as embryos of the first asexual generation. The primary masses soon lose their unity of organization and break up by constriction and separation into secondary cell masses or embryos of a second asexual generation. This happens once more and tertiary masses or embryos of the third asexual generation are formed. These tertiary embryonic masses then completely disorganize into components which are the equivalent of single germ cells, each of which goes through a typical process of embryonic development to form an adult insect. It is not always possible to trace each insect back to a single cell, but this was done in enough cases to make it practically certain that each one starts from a single cell. Such a cell is therefore essentially a precociously formed germ cell capable of parthenogenetic development. How strikingly like the liver-fluke case this is, and how unlike that of the armadillo, the reader may readily ascertain if he studies the respective life-histories side by side. Unless, therefore, we are prepared to reduce all twinning phenomena to a parity with the condition in the parasitic hymenoptera or even the liver fluke, we should cease to refer to the mode of multiple embryo formation in the armadillo as polyembryony and call it what it really is—twinning.

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